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EEG AFTER ANGIOCARDIOGRAPHY

by

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It is a well-known fact that cerebral angiography with Diodrast may sometimes give rise to cerebral complications producing, for example, hemiparesis and epileptic fits.

Broman and Olsson (1948) demonstrated in experiments in animals that contrast media of the Diodrast type are capable of impairing the vascular permeability of the cerebral vessels and they (1949) ascribed the cerebral signs to a disorder of this kind. The action of Diodrast on cerebral function in animals and man was investigated by Foltz, Thomas and Ward (1952). Continuous recording of the electroencephalogram during intracarotid injections of Diodrast 35 % and 70 % demonstrated multiple abnormalities which, according to the authors, seemed to be due to a direct toxic action on the neurones. The effects observed were divided into two groups, negative and positive. The former consisted of a flattening of all wave forms and loss of spikes, the latter in an augmentation of the delta activity and spikes, in some cases with no antecedent fast activity in the production of spikes and generalized seizure discharges. In a later paper (1954) the authors state that the most recurrent feature encountered was the augmentation of a previously present delta focus associated with local cerebral pathology. The transient flattening in human cases was usually over in a few seconds, though the recovery phase could be as long as 20 minutes. Augmentation of focal slow activity did not last more than 15 minutes after the injection. Between 1950 and 1952 Melin published several papers on the effects of angiocardiography on the electroencephalogram in children. No change was seen after venous

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angiocardiography in cases without an 'overriding' aorta. The same procedure in cases with an 'overriding' aorta, however, produced changes in 19 out of 38 patients. The changes lasted more than 48 hours. Aortography was performed in 7 cases; in 4 of these the EEG was also changed more than two days after the examination. MELIN gives no description of the types of changes. The records from two patients show, however, a decrease of the amount of alpha rhythm and a marked augmentation of slow waves. He believes that the EEG abnormalities are produced by the contrast medium, and rules out the possibility that they may have been caused by the anaesthetics given before the injection. Because of the rather high percentage of abnormalities observed by MELIN after aortography in children it was decided to perform a similar investigation in a material predominantly comprising adults.

Material and methods

The material consists of 29 consecutive cases, 19 males and 10 females. In 20 cases thoracic aortography was performed, in 7 the contrast medium was injected into the pulmonary artery, and in 2 cases into the right ventricle.

One patient was seven years old, seven patients between 11 and 17 years of age, and in the rest of the cases the age varied between 20 and 46 years.

The diagnosis in the different cases and the site of injection are shown in Table 1.

Table I

Diagnosis and site of injection in the different cases

Diagnosis	No. of cases with injection into aorta	pulm. art.	r. ventr.	Total no. of cases
Coarctation of aorta	7			7
Coarctation of aorta + aortic incomp.	2			2
Coarctation of aorta + subaortic stenosis	1			1
Op. coarctation of aorta	2	1		3
Op. coartation of aorta + aortic incomp.	3			3
Op. coarctation of aorta + atrial sept. def.	1			1
Persistent duct. arteriosus	1			1
Op. persistent duct. arteriosus	1			1
Ventricular septal defect	1	1		2
Atrial septal defect		1		1
Fallot's tetralogy			2	2
Aortic valv. disease		1		1
Mitral valv. disease		3		3
Aortic and mitral incompetence	1			1
	20	7	2	29

One hour before the catheterization the adult patients were given 5 cg of Nembutal, 0.75 cg of morphine and 0.25 mg of scopolamine; reduced doses were administered to children. The catheterizations were made under local anesthesia. The angiocardiography was performed under narcosis with controlled respiration, the narcosis consisting of the intravenous injection of pethidine hydrochloride (50 to 100 mg). In 26 cases, 1 to 1.5 ml/kg bodyweight Umbradil 70 % (a Swedish preparation with the same chemical composition as Diodrast) was injected, in the remaining three cases a corresponding quantity of triiode compounds was used (Rheopac, Triurol, and Urokon). The injection speed was 20 to 30 ml per second. The patients were awake 10 minutes after the end of the examination. No clinical complications were observed during or after the procedures.

An EEG was taken some days or hours before the roentgen examination. The patients were not fasting. The first EEG after the roentgenography was performed 19 to 20 hours later. If this record was unchanged or 'improved', no further examinations were performed; otherwise the EEG was repeated until it showed the same activity as before the angiocardiography. Recording was bipolar throughout, the Montreal 10—20 system with 21 electrodes being used in 16 cases, 19 electrodes — the National Hospital system — in 12 cases, and, finally, in one case (of the seven-year-old girl) 14 equally spaced electrodes. The total recording time was 30 minutes, including a period of over-ventilation during 3 minutes.

Results

Injection into the aorta. The EEG before injection of the contrast medium was normal or within normal limits in 11 cases, and abnormal in 9. In 8 cases the abnormality was generalized and unspecific and in 1 case unspecific but localized to the right posttemporal region.

In 16 cases the record was completely unchanged after aortography (the record before the injection in these patients was normal or within normal limits in 8 cases and abnormal in the remaining 8). The records in 4 cases were, however, changed (Table II).

Aortography thus produced a slightly decreased alpha frequency in 3 cases, and an augmentation of slow activity in 2. In 1 case a localized low voltage fast activity was evoked. The alpha frequency was restored after 3, 5, and 14 days, the slow activity had within a week been reduced to the same amount as seen before the examination, while the fast activity was only observed on the day following the injection.

Table II

Injection into the aorta

Diagnosis, age	EEG before investigation	EEG after investigation
Aortic coarct. + aortic incomp. 11 years	*Normal* Alpha fr: 9—10 c/s	1. Alpha fr: no activity at 10 c/s during 2 days 2. Low voltage 20/s activity in left frontotemp. region 1 day after inj.
Op. aortic coarct. + aortic incomp. 27 years	*Normal* Alpha fr: 10—11 c/s	Alpha fr: decrease by 1 c/s; recovery 5 days after inj.
Op. aortic coarct. 29 years	*Within normal limits* Alpha fr: 9 c/s Slight amount of 5—6/s waves in frontal areas	1. Alpha fr: no change 2. Increase of slow activity; recovery 5 days after inj.
Op. aortic coarct. + aortic incomp. 22 years	*Abnormal* Alpha fr: 9—10 c/s Scattered bil. synchr. 2—5/s waves in postcentral areas	1. Alpha fr: decrease by 1 c/s recovery 2 weeks after inj. 2. Increase of slow activity; recovery 1 week after inj.

The contrast medium Rheopac was used in one of the 4 cases in which the records were changed (a 22-year-old patient operated on for coarctation of the aorta and with additional aortic incompensation). Urocon and Triurol were injected in 2 cases in which the records were unchanged, while Umbradil was used in the remaining 17 cases.

Injection into the pulmonary artery. In 4 cases the EEG was either normal or within normal limits before the injection. The remaining 3 cases had abnormal records, showing in 1 case bilaterally synchronous high voltage 6/sec waves from the frontal regions, in another case episodes of high voltage 4/sec waves from all areas, and in the third case single discharges of paroxysmal high voltage 3 to 5/sec waves from both temporal regions.

Following the injection the records were changed in 4 of these 7 cases (Table III).

The Contrast medium Umbradil was administered in all the cases. As seen from the table the injection brought about a decrease of the alpha frequency by half a cycle per second in 1 case; in another case a slight increase of slow activity was recorded. In both patients the changes had disappeared after 2 days. In 2 cases, on the other hand, a reduction of high voltage discharges was observed on the day after the roentgenography.

Table III

Injection into the pulmonary artery and right ventricle

Diagnosis, age	EEG before investigation	EEG after investigation
Mitral stenosis + incomp. 33 years	*Normal* Alpha fr: 10.5 c/s	Alpha fr: decrease by 0.5 c/s; recovery 2 days after inj.
Atrial septal defect. 40 years	*Within normal limits* Alpha fr: 8—10 c/s Slight amount of 5—7/s waves in all regions	1. Alpha fr: no change 2. Slight increase of slow activity; recovery 2 days after inj.
Op. aortic coarct. 7 years	*Abnormal* Alpha fr: 8—9 c/s Episodes of high voltage 4/s waves	1. Alpha fr: no change 2. Decrease of paroxysmal activity on day following inj.; 'recovery' 2 days after inj.
Mitral stenosis. 41 years	*Abnormal* Alpha fr: 11 c/s Discharges of high voltage 4—6/s waves from temp. regions	1. Alpha fr: no change 2. No high voltage discharges on day following inj.; no further records taken
Fallot's tetralogy. 26 years	*Abnormal* Alpha fr: 8 c/s Episodes of mod. voltage 7/s waves	1. Alpha fr: no change 2. Decrease of episodic activity on day following inj.; no further records taken

Injection into the right ventricle. This procedure was carried out in 2 cases. One of the cases had a normal record before the injection; there was no change after this procedure. In the other case the first record showed a generalized unspecific abnormality with episodes of moderate voltage 7/sec waves from all regions. Only one record was taken after the injection of the medium, and this electroencephalogram showed a reduced amount of theta activity.

Discussion

Previous investigations have shown that cerebral angiography with contrast media of the Diodrast type may give rise to brain lesions. The purpose of this investigation was to find out if angiocardiography with the technique used in our hospital brought about any severe and long-standing cerebral damage revealed by electroencephalography. A comparison of the EEGs before, and 19 to 20 hours after angiocardiography showed that this was not the case. Thus, aortography produced a slight

slowing of the activity in 4 cases out of 20 while injection of the contrast medium into the pulmonary artery gave rise to similar changes in 2 cases out of 7. In 5 cases these changes had disappeared within a week, in 1 case after 14 days. It was impossible to perform daily EEG recordings after the angiocardiographies due to practical difficulties. If, however, this had been done one might possibly have observed an earlier recovery in some cases. In the case in which the changes had not disappeared after a week, there was an interval of seven days between the last EEG in which the alpha frequency was still reduced and the next recording (2 weeks after angiocardiography) with complete recovery.

One may of course ask to what extent the EEG abnormalities had been caused by the narcosis or the anaesthetic technique. The EEG changes seen after superficial narcosis are due to the specific effects of the anaesthetics on the brain. In deep narcosis one has also to take inot account that a certain degree of anoxia may have been present. In the present investigation the patients were awake 10 minutes after the angiocardiography. There was in no case deep or prolonged narcosis and the changes of the electroencephalograms were consequently not due to anoxia. It is also unlikely that the slowing of the activity was evoked either by Pethidin or Pentothal as both substances have a very transitory effect on the brain. (GOODMAN and GILMAN, PAMPIGLIONE). One may thus draw the conclusion that the changes in the electric activity of the brain observed in our material were due solely to the action of the contrast media. In 3 cases a diminution of episodic and paroxysmal activity was seen after angiocardiography; further EEG recordings were unfortunately only performed in 1 of these. This decrease of abnormality may have been caused by the contrast medium — FOLTZ et coll. observed a flattening of the activity in some cases. It may also have been due to chance as it is a wellknown fact that episodic and paroxysmal activity may change from one day to another in the same subject.

MELIN claimed that aortography in his material brought about changes in 4 cases out of 7. One reason for the discrepancy between the large percentage of abnormalities in his material and the rather insignificant changes in the present investigation may be that his material exclusively comprised children while ours predominantly consisted of adults. It is admitted that the immature brain is very susceptible to brain injuries and also that the EEG in children often takes longer time to stabilize than does that of the adult following injury. Another explanation to the frequently observed abnormalities in his material may be that the narcosis was deep and of long duration. If this was the case, the pathologic activity, encountered in such a high a percentage of cases in his material, may also partly have been due to anoxia.

SUMMARY

Twenty-nine mainly adult cases were examined by EEG before and after angiocardiography; in 20 the contrast medium was injected into the ascending aorta and in the remainder, the injection was made into the pulmonary artery or right ventricle. Slowing of the activity was observed in 6 cases, with return to a normal record within 2 to 14 days after the injection; in another 3, a reduction of paroxysmal activity was seen, and in 20 cases the EEG was completely unchanged. The influences of the anesthesia is discussed.

ZUSAMMENFASSUNG

Elektroencephalographische Untersuchungen wurden vor und nach der Angiokardiographie in 29 Fällen von hauptsächlich Erwachsenen ausgeführt; in 20 Fällen wurde das Kontrastmittel in die Aorta ascendens injiziert, in den übrigen wurde die Injektion in die Arteria pulmonalis oder in den rechten Ventrikel gemacht. Verlangsamung der Aktivität wurde in 6 Fällen beobachtet, bei denen innerhalb von 2 bis 14 Tagen der normale Verlauf wiederkehrte. In weiteren 3 Fällen wurde eine Reduktion der paroxysmalen Aktivität beobachtet und in 20 Fällen war das Elektroenzephalogramm völlig unverändert. Der Einfluss der Anästhesie wird diskutiert.

RÉSUMÉ

Vingt-neuf malades, en majorité des adultes, ont subi un examen EEG avant et après angiocardiographie; dans 20 cas, le produit de contraste a été injecté dans l'aorte ascendante; dans les autres, l'injection fut faite dans l'artère pulmonaire ou le ventricule droit. On a observé un ralentissement de l'activité dans 6 cas, avec retour à un tracé normal de 2 à 14 jours après l'injection; dans 3 autres cas il y eut une réduction de l'activité paroxystique et dans 20 cas l'EEG resta absolument inchangé. Les auteurs examinent l'influence de l'anesthésie.

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PULMONARY CHANGES FOLLOWING BRONCHOGRAPHY WITH DIONOSIL OILY (ANIMAL EXPERIMENTS)

by

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The first contrast media to be used in bronchography were iodized oils, such as Lipiodol and Jodipin. In these preparations the iodine is bound to unsaturated fatty acids of poppy seed oil and sesame oil. These media have physical and biologic drawbacks; the greatest disadvantage is the retention of oil owing to its poor absorbability, disturbing repeat examinations and, more important, resulting in chronic irritation of the lung tissue, with formation of iodine granulomata and even progressive pulmonary fibrosis. FISCHER reports that retention of oil for more than 90 days is regularly followed by granulomata and pulmonary lesions.

The iodized oils were replaced by water-soluble viscous contrast media such as 'Umbradil Viskös B' and 'Joduron B'. However, these too proved to be unsatisfactory owing to the irritation and granulomata caused by the viscosity-increasing factor carboxymethyl cellulose (BJÖRK and LODIN, WEBER, WERTHEMANN and VISCHER, VISCHER).

The most recent media on the market are based on the suspension principle.

In the case of Lipiodol-sulphanilamide the sulphanilamide is suspended in powder form in the oil in order to raise the viscosity; the absorbability is probably similar to that of Lipiodol. With Dionosil Aqueous and Oily the opaque substance is suspended in water or ground nut oil; in contrast to Lipiodol and Jodipin the iodine is not combined with the oil. The oil in Dionosil is said (STUTZ and VIETEN) to be broken down into glycerine and "species-specific" fatty acids the absorbability of which is supposed to be much greater than that of the break-down

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products of the earlier iodized oils, *viz.* glycerine and iodized foreign unsaturated fatty acids.

As far as we have been able to ascertain, no investigation into the absorbability of Dionosil Oily has been reported. The opaque substance is absorbed and is as a rule excreted by the kidneys within a week of administration; it was with a view to elucidate the absorption and local effect upon the lung tissue of the oily component that the present investigation was carried out.

Technique. Bronchography was carried out by percutaneous endotracheal injection of Dionosil Oily under barbiturate anaesthesia in 20 rabbits weighing about 2,500 g. Filling of the bronchi was confirmed by roentgenograms; about 1 ml of the contrast medium was enough to give satisfactory filling.

All animals including 10 controls were kept under identical conditions.

Histologic examination of the lung tissue for retention of fatty substances and parenchymatous lesions was made 3 months after bronchography in half of the experimental series and 6 months after bronchography in the other half, as well as in the controls.

Results

3-months group. Oil residua were found in 8 of the 10 animals, the amounts being considerable in 4 (Fig. 1 a). In 3 of these 8 a few small foreign-body granulomata were present.

6-months group. Oil residua were demonstrable in 7 of the 10 animals. Foreign-body granulomata were found in all 7 animals. They were larger and more numerous than in the 3-months group (Fig. 1 b).

Both the 3-months and 6-months groups showed general thickening of the septa and alveolar walls. Oil was present in the alveoli, alveolar walls, lymph-vessels, and in some of the granulomata.

Controls. No pathologic changes were found (Fig. 1 c).

Discussion

It is shown that bronchography with Dionosil Oily in the rabbit is followed by considerable retention of the oily component after as much as 6 months, with areas of pathologic changes in the lung tissue which, as far as granulomata is concerned, are greater and more numerous after 6 than after 3 months. These observations accord with those of FISCHER concerning the earlier iodized oils, viz. parenchymatous changes supervene when oil is retained for more than 90 days.

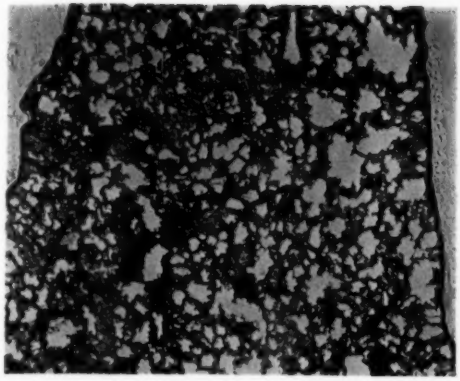

Fig. 1. a) Oil residua 3 months after bronchography with Dionosil Oily.

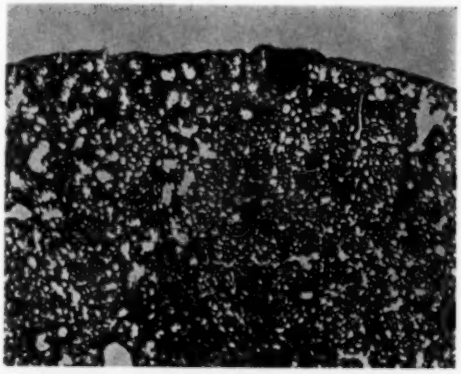

Fig. 1 b) Granulomata and oil residua 6 months after bronchography with Dionosil Oily.

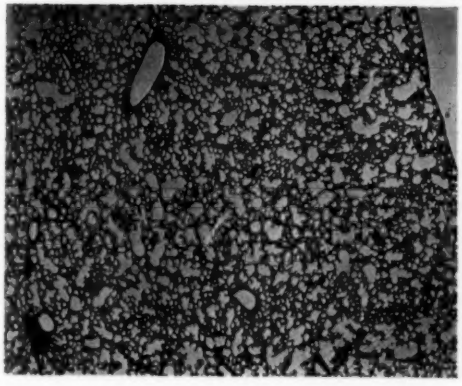

Fig. 1 c) Control. Normal lung.

The difference in chemical structure between the iodized oils and Dionosil Oily would therefore seem to be of no significance with regard to the elimination of the oily component. The preparations appear to have the same effects on the lung tissue.

The difficulties in studying the absorption of such substances in man are obvious, particularly in normal subjects. In a few operation specimens obtained not longer than 3 weeks after bronchography, oil residua, sometimes considerable, have been demonstrated. In such cases, however, parenchymatous lesions were already present, and the means of elimination clearly much impaired.

Conclusions

In so far as results of animal experiments such as these can lead to conclusions applicable in man the following may be said. Oily suspensions of Dionosil should be abandoned in favour of the aqueous suspension, which furthermore is easier to handle. If it is nevertheless decided to use the oily suspension, as much as possible should be aspirated immediately after bronchography, and 'peripheral filling' scrupulously avoided, since the oil is chiefly eliminated via the air-passages.

The aqueous suspension of Dionosil contains the viscosity-raising factor carboxymethyl cellulose, but in smaller amounts than the water-soluble, viscous contrast media. In 4 rabbits we were unable to detect parenchymatous changes 4 months after bronchography with the aqueous suspension, and it is our opinion that this constitutes the best contrast medium for bronchography at present available.

SUMMARY

Bronchography was carried out with Dionosil Oily in rabbits. After 3 months, oil residua were found in 8 of 10 animals, after 6 months in 7 of 10 animals. Foreign body granulomata were more numerous in the 6-months group. Dionosil Oily should be abandoned in favour of the aqueous suspension of Dionosil.

ZUSAMMENFASSUNG

Bronchographie mit in Öl aufgelöstem Dionosil wurde bei Kaninchen ausgeführt. Nach Verlauf von 3 Monaten fand man Ölreste bei 8 von 10 Tieren, nach 6 Monaten bei 7 von 10 Tieren. Fremdkörpergranulome waren in der 6-Monategruppe zahlreicher. Die ölige Dionosillösung sollte zu Gunsten der wasserlöslichen Dionosilsuspension verlassen werden.

RÉSUMÉ

Les auteurs ont fait sur des lapins des bronchographies avec du Dionosil Huileux. Ils ont trouvé des résidus huileux au bout de 3 mois chez 8 animaux sur 10, au bout de 6 mois chez 7 animaux sur 10. Les granulomes à corps étrangers étaient plus nombreux dans le groupe examiné au bout de 6 mois. Le Dionosil Huileux devrait être abandonné au profit d'une suspension aqueuse de Dionosil.

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EEG DURING CEREBRAL ANGIOGRAPHY

by

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A number of clinical and experimental observations have demonstrated that contrast media of the diodrast type may produce cerebral vasomotor phenomena (HOLM 1944; FOLTZ et coll. 1952; INGVAR & SÖDERBERG 1957; GREITZ 1956). It has also recently been shown that an intimate correlation exists between the EEG and the cerebral vasomotor tone (INGVAR & SÖDERBERG 1956). The present investigation was therefore carried out in order to determine whether contrast injections during various forms of routine cerebral angiography will produce any acute changes in the EEG. In the 17 cases investigated no such changes were found.

Material and methods. EEG recordings were made in 15 cases admitted to the Neurosurgical Department. Two (2) cases of congenital heart disease were also examined. A routine EEG was made prior to the angiographic examination. One case was investigated both during carotid and vertebral angiography. Continuous EEG recordings were made in the roentgen room during the procedures. The EEG was followed for several hours after the termination of the injections and was repeated towards the end of the day and, in the majority of cases, on the following day.

Recordings were performed with a six-channel Grass Model III EEG apparatus with ordinary silver plate electrodes fastened to the scalp with collodium. During the injections bipolar leads of a type which covered both hemispheres symmetrically were employed. In most cases interference from the roentgen equipment was found to be very small provided earthing had been properly carried out.

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The material consisted of 6 cases of subarachnoid haemorrhage, 4 cases of cerebral tumour, 3 cases of epilepsy, and 1 case of increased intracranial pressure. All the cases, except the two of congenital heart disease, were examined unanaesthetized. Routine premedication (0.01 g morphine) before the angiography was usually employed; papaverine was avoided. The patients were all conscious during the examinations. For carotid angiography (9 cases) the percutaneous technique was used and for vertebral angiography (6 cases) the catheter method of RADNER (1951). A contrast medium of the diodrast type, Umbradil 35 %, injected in doses of 10 ml at a time was employed for the cerebral angiographies. This medium is being used routinely in the department since BROMAN & OLSSON (1949) demonstrated experimentally that it causes less damage to the blood brain barrier than other contrast media of the diodrast type. The patients received between 2 and 5 doses of 10 ml. For cardioangiography, 20 ml Umbradil 70 % were injected through a catheter into the left side of the heart. The injection time was 2 seconds.

Results. There were no clinical signs of neurologic complications arising from the angiographic procedures. Subjectively the patients experienced the usual sense of heat or moderate acute pain in the scalp and eye on the side injected.

In none of the 17 cases investigated did the EEG show any acute changes during or immediately after the injections of Umbradil. This applied both to the neurosurgic cases as well as to the two cases of congenital heart disease investigated under anesthesia with higher concentration of contrast medium. Careful inspection of the records did not reveal any acute slowing of the main dominant frequencies. If the EEG before angiography was normal it remained so and no acute activation was seen of epileptic or other EEG abnormalities existing prior to the angiographic examinations.

In confirmation of what has been demonstrated before by other workers (FOLTZ et coll. 1952) a slight increase of previously existing slow waves (delta activity) was found in three cases. This increase, however, did not appear until a few hours or the day following the angiography.

Discussion

The results indicate that the cerebral vasomotor changes which can be assumed to accompany cerebral angiography (INGVAR & SÖDERBERG 1957, et alios) do not influence the electric activity of the brain. This apparently holds true both for angiographic procedures which mainly involve the hemispheres (carotid angiography), as well as for vertebral angiography in which the major part of the brain stem is involved. In this respect the present results are more favorable from a clinical point

of view than those of FOLTZ et coll. (1952) who also studied the EEG in animals and man during diodrast injections into the carotid artery. Their impression was that diodrast might seriously endanger cerebral functions and that EEG changes after angiography were relatively common. MELIN (1952) has also pointed out that EEG changes are not infrequent in children after cardioangiography.

The findings presented here are more in agreement with those of KIRSTEIN et coll. (1956) who studied the EEG in 29 patients after angiocardiography performed under general anaesthesia. They only found a mild but transient slowing of the dominant rhythms in 6 cases.

The investigation thus does not support the view that routine cerebral angiography as a rule influences cerebral functions harmfully. On the other hand, the general impression is confirmed that patients with some local or general disturbance of cerebral circulation existing prior to the angiography may tolerate the procedure less well (OLSSON 1949). This is concomitant with the fact that in three cases, in which focal slow waves were found in the first EEG there was a slight increase of the pathological activity after angiography.

SUMMARY

Serial EEG examinations were made in 17 cases during various forms of routine cerebral angiographies in order to determine whether any alterations could be recorded. No acute changes were observed but in 3 cases a slight increase of previously existing slow waves occurred several hours after the examination.

ZUSAMMENFASSUNG

Elektroenzephalographische Reihenuntersuchungen wurden in 17 Fällen bei verschiedenen Formen rutinmässiger cerebraler Angiographien ausgeführt. Die Absicht war zu entscheiden, ob irgendwelche Veränderungen registriert werden können. Akute Veränderungen wurden nicht beobachtet; in drei Fällen jedoch trat mehrere Stunden nach der Untersuchung eine leichte Erhöhung einer bereits vorher sichtbaren schwachen Welle auf.

RÉSUMÉ

L'auteur a fait des examens EEG en série dans 17 cas pendant différents types d'angiographies cérébrales ordinaires pour voir s'il était possible d'enregistrer des modifications. Il n'a pas observé de modifications importantes, mais, dans 3 cas il a constaté, plusieurs heures aprés l'examen, une légère augmentation d'ondes lentes qui existaient auparavant.

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FROM THE NOBEL INSTITUTE FOR NEUROPHYSIOLOGY (DIRECTOR: PROF. RAGNAR GRANIT), KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN

CEREBRAL VASOMOTOR TONE AND EEG DURING INJECTIONS OF UMBRADIL.

An experimental study with a new method

by

David H. Ingvar and *Ulf Söderberg*

There is at present no definite agreement in the literature as to the vasomotor effects of cerebral angiographic procedures with contrast media of the diodrast type (GREITZ 1956). Experimental studies of the cerebral circulation during injections of radiopaque agents have mostly been made by direct observation of pial vessels on the exposed brain surface, often with the aid of the cranial window technique. Such methods are obviously limited to surface vessels and they also lack sufficient sensitivity to determine small caliber changes.

In the present study a new sensitive and continous method has been used for measuring cerebral circulation experimentally (INGVAR & SÖDERBERG 1956) together with registration of the EEG and the systemic blood pressure as well as other variables. The technique used has proved to be useful for studies of the cerebral effects of contrast media. It will be shown that in the cat a commonly used contrast medium of the diodrast type, Umbradil, invariably induces an initial vasoconstriction, often followed by a long lasting vasodilatation, of the cerebral vessels both of which must chiefly be due to its local action upon the vessels.

Methods. Anaesthetized cats under Nembutal (40 mg/kg intraperitoneally) as well as *encéphale isolé* animals, prepared under ether or Pentothal, were used.

Our method of studying the cerebral blood flow involves the measurement of the total venous outflow from the cannulated sagittal sinus after

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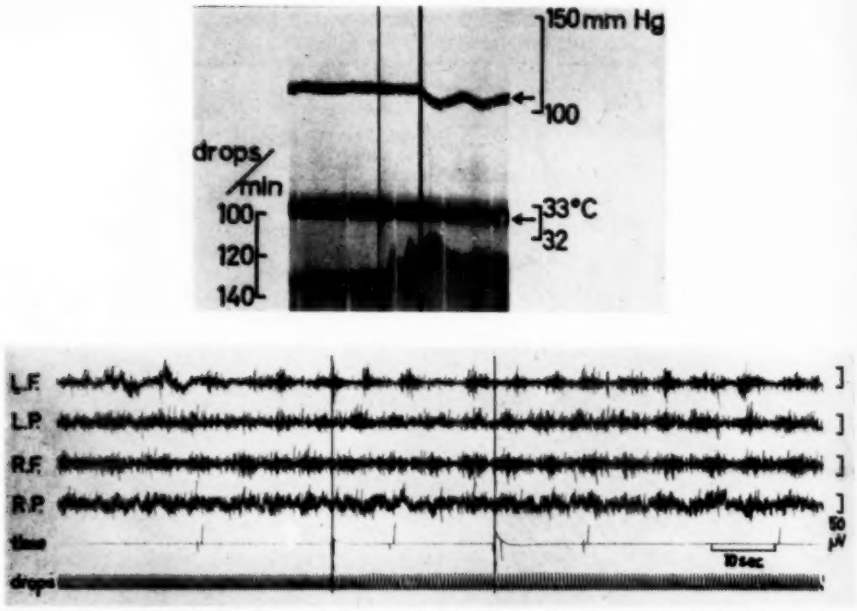

Fig. 1. Cat, 3.0 kg. Light Pentothal anaesthesia. *Upper records:* Systemic blood pressure, ear skin temperature, and cerebral blood flow. *Lower records:* EEG from four cortical areas. (L = left; R = right; F = frontal; P = parietal.) Time markings every 30 sec (corresponding to interruptions of upper diagram) and blood drops, the time intervals of which are represented as thin vertical lines in upper flow record. Between thick vertical lines 0.5 ml Umbradil 35 % injected into left carotid artery. Note: Transient reduction of cerebral blood flow, appearing about 30 sec before decrease in blood pressure. No effect on EEG.

the anastomoses to the diploic veins has been eliminated. An electric drop recorder was used for measuring the flow from the cannula. In most experiments the craniotomy over the sagittal sinus was closed by dental acrylate resin in order to restore intracranial pressure conditions.

The blood pressure was measured in the femoral artery with an electromanometer (ELEMA, Stockholm). In some experiments the ear skin temperature was followed as an index of cutaneous circulatory conditions.

Rapid injections of Umbradil (Diodone, the diethanolamine salt of 3,5-diiodo-4-pyridone-N-acetic acid; 0.15 to 1.0 ml/kg in concentrations of 35, 50 and 70 per cent at room temperature) were made into the carotid artery by means of a thin polyethylene catheter inserted into the central end of the cut lingual artery. Most intracarotid injections were made with a dose of 0.15 ml/kg which in relation to body weight roughly cor-

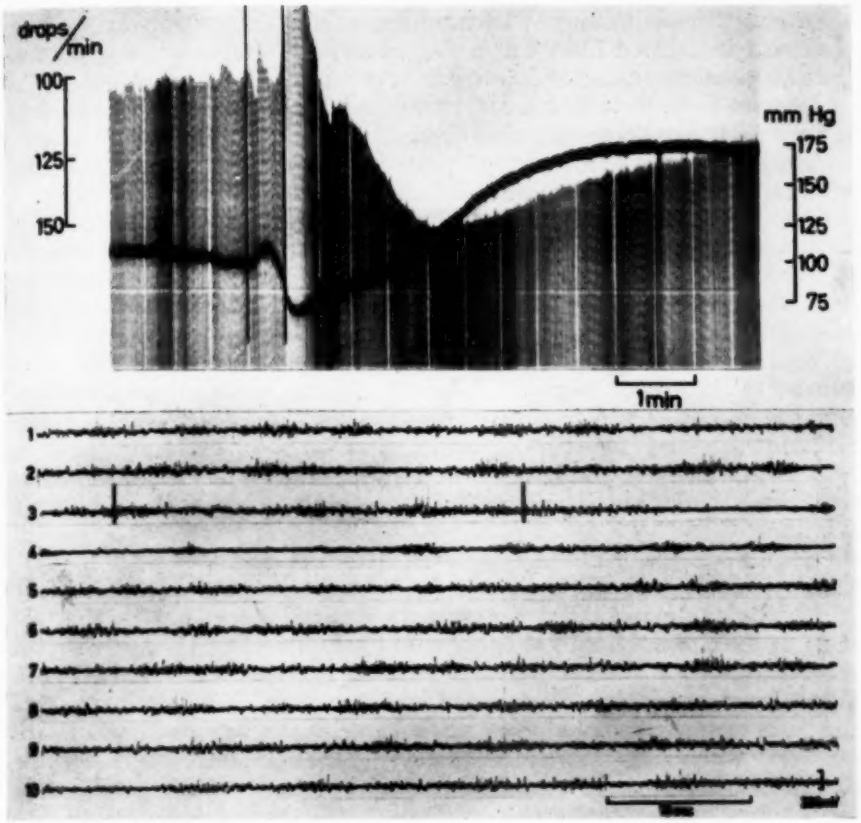

Fig. 2. Cat, 2.6 kg. Nembutal. Cerebral blood flow and arterial blood pressure (*upper part*). 10 consecutive tracings of EEG from left suprasylvian gyrus (*lower part*). Between thick vertical lines in both top and bottom records 5 ml Umbradil 70 % injected into femoral vein. Slight increase in blood pressure and small decrease in cerebral blood flow during period of injection. Immediately after injection marked decrease in flow appears concomitantly with reduced blood pressure, followed by long period of increased blood pressure with a transient reduction of cerebral vasomotor tone. EEG shows decreased cortical activity during first minute after injection.

responds to the single dose used routinely in clinical cerebral arteriography. Some injections were made into the femoral vein.

The EEG was recorded with bipolar silver ball electrodes mounted in drill holes with dental acrylate. A six-channel electroencephalograph (Offner, Type A) was used.

Results. The injections of Umbradil invariably gave a rapid reduction of the cerebral blood flow, which started a few seconds after the onset of the injection into the carotid artery. The cerebral blood flow was observed to decrease from 10 to 25 per cent. The effect seemed roughly proportional to the amount and concentration given. A typical result is shown in Fig. 1 in which the vasomotor change precedes the effects on the systemic circulation by several seconds. With succeeding injections there was a progressive reduction in the degree of vasoconstriction produced. After several repeated intracarotid injections, even with small doses, a state of lowered vascular resistance was sometimes obtained, *i. e.* the blood flow from the brain remained increased in spite of the fact that the blood pressure returned to the level before the injection.

In many cases, especially with large doses, a marked and long-lasting increase of the blood pressure followed (Fig. 2) and the ear skin temperature was observed to drop, indicating a subcutaneous vasoconstriction.

The effects obtained seemed to be independent of the anaesthetic level since similar results were seen in *encéphale isolé* and in barbiturate animals.

Section of the cervical sympathetic trunks and the vagi had no observable influence upon the results.

EEG recordings taken continuously during the injections did not show any changes of a local or general character after the intracarotid administration of small doses of Umbradil in spite of the fact that a reduction of the cerebral flow was always seen (Fig. 1). With larger doses and higher concentrations some EEG disturbances were recorded which coincided with larger changes in the systemic circulation (Fig. 2). These EEG disturbances were probably caused by the circulatory failure in combination with local vascular factors (Foltz et coll. 1952). The EEG was not followed for longer periods after the injections.

Discussion

The decrease in cerebral blood flow after injections of Umbradil into the carotid artery of the cat can only be due to cerebral vasoconstriction since there was no simultaneous reduction in the systemic blood pressure. This vasoconstriction was caused by a direct effect on the blood vessels, as procedures influencing the regulation of vasomotor tone, such as changing the depth of anaesthesia or sectioning the spinal cord, the vagi, and the cervical sympathetic trunks, were all without effect upon the results.

Umbradil has a high viscosity. A mere increase in the viscosity of the blood passing through the brain can, however, hardly explain the

decrease in flow since the effect of the injections were less when repeated several times and were not even noticeable when damage of the walls of the vessels had definitely occurred. Support for the assumption of cerebral vasoconstriction was also obtained in the general increase in vasomotor tone seen after intravenous injections of larger doses of the contrast medium (Fig. 2).

Local irritative effects of contrast media have earlier been repeatedly demonstrated, mainly by BROMAN & OLSSON (1948, 1949, 1951) in their extensive studies of the effect of diodrast compounds on the blood-brain barrier (cf. also BASSETT et coll. 1953).

The present results confirm the findings of cerebral vasospasm after intracarotid diodrast injections reported by FOLTZ et coll. (1952). The fact that SCHMIDT (1955) as well as BROWNE & STERN (1954), except in one single observation, were unable to find signs of vasoconstriction after diodrast may be explained as due to species differences, chemical variations of the compounds used, as well as other technical divergencies. The effects of small doses of Umbradil were, as stated above, also rather small and a method with high sensitivity which follows rapid changes is necessary for detecting them.

BLOOR et coll. (1954) investigated the effects of Iodopyracet upon the cerebral blood flow measured with a thermoelectric method. They concluded that the principal reaction of the cerebral vessels to diodrast was vasodilatation. In about 40 per cent of the experiments, however, they obtained an initial transient vasoconstriction and it would seem that the vasodilatation which followed could to some extent have been caused by local irritative effects (cf. above).

The slight but long lasting decrease in cerebral vasomotor tone seen after repeated injections of Umbradil may be explained as a reactive process, secondary to the repeated injury of the vessel walls (BROMAN, FORSSMAN & OLSSON 1950). It should, however, be pointed out that no remarkable abnormalities of the EEG were seen during that phase. In one case, however, the EEG showed a pattern of moderate arousal during the period of vasodilatation.

The EEG records do not support the view that EEG changes are frequent or severe after injections of doses of Umbradil comparable to those used clinically. From previous experiments of a similar type (FOLTZ et coll. 1952) the impression is obtained that EEG complications to contrast injections into the carotid artery are common and often very severe. The workers mentioned, however, as a rule used larger amounts and higher concentrations of contrast fluid. FOLTZ et coll. (1952) reported a 'flattening reaction' of the EEG during diodrast injections. In our experience such reactions occurred during the transient failure of the systematic circulation which followed administration of large amounts of Umbradil (Fig. 2).

The present EEG observations during contrast injections in animals are in accordance with the clinical observations of INGVAR (1957) who continuously followed the EEG in 15 patients before, during, and after various forms of routine cerebral angiography. In this series there were no acute changes recorded in the EEG. KIRSTEIN et coll. (1957) reported similar results after angiocardiography.

The results presented have thus not given experimental evidence for the view that Umbradil, in doses and concentrations comparable to those employed in clinical cerebral angiography, has any important injurious effect upon the central nervous system as far as can be judged from the EEG. This conclusion seems to be valid in spite of the fact that cerebral vasomotor changes invariably were recorded after injections of small doses.

The authors are indebted to the Swedish Medical Research Council for financial support. Umbradil was kindly provided by AB. Astra.

SUMMARY

Umbradil was injected into the carotid artery or femoral vein in unanaesthetized or anaesthetized cats. The cerebral blood flow was continuously recorded with a new method concomitantly with the EEG and the systemic blood pressure. Initial cerebral vasoconstriction invariably occurred as well as subsequent vasodilation after repeated injections; with doses comparable to those used clinically no changes were seen in the EEG.

ZUSAMMENFASSUNG

Umbradil wurde in die Arteria carotis oder in die Vena femoralis nicht narkotisierter oder narkotisierter Katzen injiziert. Die cerebrale Durchblutung wurde fortlaufend mit einer neuen Methode gleichzeitig mit dem EEG und dem Blutdruck gemessen. Initiale cerebrale Gefässverengerung und nachfolgende Gefässerweiterung trat nach wiederholten Injektion ständig auf. Mit Kontrastdosen, die mit den klinisch angewendeten vergleichbar sind, wurden keinerlei Veränderungen im EEG gesehen.

RÉSUMÉ

De l'Umbradil a été injecté dans l'artére carotide ou dans la veine fémorale de chats non anesthésiés ou anesthésiés. Le débit sanguin cérébral a été enregistré de façon continue grâce à une nouvelle méthode, en même temps que l'EEG et la pression sanguine systémique. Des injections répétées ont été invariablement suivies d'une vasoconstriction cérébrale initiale puis d'une vasodilatation; des doses comparables à celles utilisées en clinique n'ont pas entraîné de modifications de l'EEG.

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EFFECTIVENESS OF UROLOGIC CONTRAST MEDIA. COMPARISON BETWEEN DIODONE AND TRIIODYL (SODIUM ACETRIOZOATE)

by

Erik Madsen

Since the introduction of urography by OSBORNE et coll. in 1923 a number of contrast media of increasing effectiveness have seen the light of day. After the war Diodone was accepted practically everywhere as the best medium, and gradually has been supplied with various admixtures to reduce its side-effects.

In 1950 Mallinckrodt Chemical Works in U. S. A. produced a new contrast medium, sodium acetriozoate, with the name Urokon, and since 1954 this medium has been manufactured in Denmark by Lundbeck & Co. under the trade name of Triiodyl. This substance is the sodium salt of 3-acetylamino-2,4,6-triiodobenzoate. Diodone consists of 3,5-diiodo-4-pyridone-N-acetic acid diethanolamine. Thus, Triiodyl carries 3 atoms of iodine in the molecule and Diodone 2. The molecular weight of Triiodyl is 579, while that of Diodone is 477. Triiodyl contains 65.8 % iodine per molecule, Diodone 55 %.

The first clinical studies were carried out by NESBIT & LAPIDES who reported that Urokon gave equally good urographic results as other contrast media. RICHARDSON & ROSE, BARRY & ROSE as well as ROBBINS et coll. have reported a more rapid excretion of Urokon than of other contrast media. This result they base on the clinical assessment of a very large number of urograms. Others are more sceptical, however. CAVE et coll., for instance, found that in 113 patients receiving 20 ml Urokon 30 % the density of the contrast medium as well as the excretion

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did not differ from those obtained with Diodone; no comparative investigations were, however, carried out in these cases. KEATES, on the other hand, has published an investigation of 32 patients injected once with Diodone 35 % and once with Urokon 30 % and found better filling of the calyces after Diodone in two-thirds of the cases.

Only a few chemical investigations are available. Studying the excretory rate in 3 cases BARRY & ROSE found that the greater part of Urokon is excreted within the first six hours. In this connection special interest attaches to a study by NEUHAUS et coll. who compared the excretion of Diodone and Urokon in 3 cases by collecting the urine by catheterization at 30-minute intervals for 3 hours after the injection. At the end of the period one patient had excreted the same amount of both contrast media, whereas in the other two the amount of Diodone excreted was definitely larger. These authors conclude that the rate of excretion varied considerably in these patients who are stated to have been suffering from renal dysfunction. On the other hand, they do not dwell on the fact that the table shows that the amount of Diodone excreted by far exceeds that of Urokon during the first half-hour after the injection.

According to the manufacturers Triiodyl is said to give a better shadow density owing to its higher content of iodine. It is particularly emphasized that the excretion is more rapid, so that exposures may be made sooner than after Diodone and the investigation completed in a shorter time. We therefore felt obliged to give the new medium a trial at our hospital.

For quite a long period we used both Diodone and Triiodyl for urographic studies, and patients for repeat investigations were given the two contrast media alternatively. Evaluation of the urograms, however, failed to confirm the alleged advantages. It even showed that in most cases the urograms were better with Diodone than with Triiodyl, the excretion usually being more rapid, the filling of the calyces better, and the density of shadows equally good.

As this result was in conflict with most other reports, we wished to support our radiologic impressions with chemical estimations. (These estimations were performed at the Laboratory of Lundbeck & Co. In the case of Diodone as well as Triiodyl the chemical method consisted of the addition of saturated bromine water to the specimen of urine and titration with sodium thiosulphate.) Unlike previous authors, we set great value upon comparing the excretion of the two contrast media during the first 30 minutes after injection, *viz.* the ordinary period of the urographic investigation.

A total of 10 normal subjects were submitted to urography on two occasions, once with 20 ml Diodone 50 % and once with 20 ml Triiodyl 50 % at an interval of at least two days. The subjects were prepared in the usual way, being deprived of solid food as well as fluid for 14 hours

before the investigation. In order not to introduce a possible variable, abdominal compression was not used. During each investigation a catheter was inserted into the bladder, and the urine collected at 5-minute intervals for 30 minutes after the injection. The quantity and iodine concentration of each specimen was then determined.

Diagram 1 sets out the average concentration of iodine measured in mg iodine/ml urine. During the entire period of the investigation it was somewhat higher with Diodone than with Triiodyl, but otherwise the two curves show a uniform course. The maximum concentration of iodine occurred during the interval 20 to 25 minutes in the case of both contrast media.

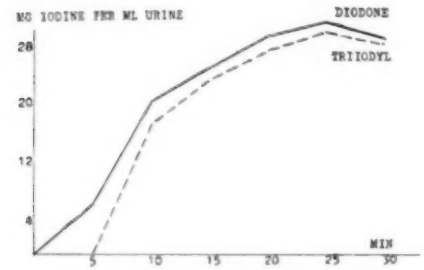

Diagram 1. Average iodine concentration in 10 subjects measured in mg iodine/ml urine.

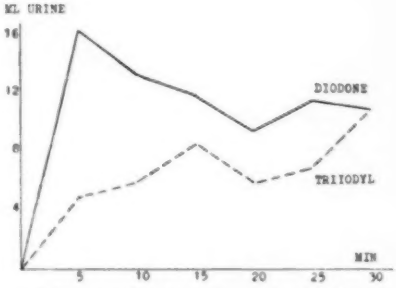

Diagram 2. Average output of urine in the 10 subjects.

The average urinary output is shown in Diagram 2. It will be seen that during the entire period of the investigation the output after Diodone exceeded that following Triiodyl. The maximum output after Diodone occurred as early as the 0—5 minute interval; after Triiodyl it did not occur until towards the end of the investigation.

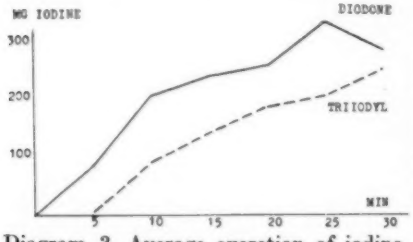

Diagram 3. Average excretion of iodine in the 10 subjects.

Comparing the two quantities: iodine concentration and urinary output, we can determine the amount of iodine excreted during each 5-minute interval as shown in Diagram 3. As might be expected the excretion of iodine after Diodone is on the average higher than after Triiodyl during the entire period. The summit of the Diodone curve is 25 minutes after the injection, whereas the Triiodyl curve increases evenly during the 30 minutes. The essential finding is, however, that with Diodone the excretion of iodine exceeds that with

Triiodyl by 30 to 100 % during the entire period as well as during each 5-minute interval.

The general procedure of testing contrast media clinically and evaluating them from the quality of the urograms has proved inadequate in the case of Triiodyl. This is not at all surprising. As a rule, subjects to be examined represent such heterogeneous conditions for excretion and technique of exposures that these factors alone make an evaluation illusory. It is better, but probably not sufficient, to submit the same patient to urography on two occasions within a short period. The only reliable basis is afforded by precise clinical and laboratory studies of the nature described above.

In the present study the chemical estimations unequivocally supported our radiologic assessment as to the diagnostic value of the two contrast media. Although regard must be paid not only to the glomerular infiltration, but also to a possible difference in the tubular excretion of the two contrast media, it must be presumed — as pointed out by KEATES — that the diuresis following intravenous injection of urographic media is due to the osmotic effect of the high concentration they reach in the urine. Better filling of the calyces with Diodone would thus have to be due to its higher diuretic effect — explained by the difference in molecular weight betweeen the two contrast media. When the two media are injected in the same concentration, the lower molecular weight of Diodone will cause more molecules to pass out into the tubules which makes the osmotic pressure higher, and this in turn will increase the urinary output.

In consequence, if Triiodyl is to be used, the ordinary diagnostic technique must be altered in order to obtain better renal filling. This was shown by PORPORIS, ZINK, WILSON & BARRY who obtained improved filling by allowing the patients to drink water half an hour before the investigation.

Supplementary experiments after administration of water. In an attempt to investigate this procedure, we gave two subjects — prepared in the usual way by dehydration for 14 hours before examination — 200 g of water

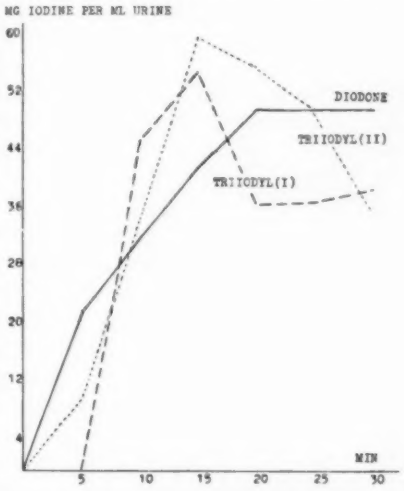

Diagram 4. Iodine concentration in one subject after administration of water and Triiodyl (II) compared with the iodine concentration with Diodone and Triiodyl (I) after usual dehydration.

by mouth half an hour before the injection of Triiodyl. In both instances, the urograms showed equally good filling of the calyces as with Diodone. In one of these cases the iodine concentration and the output of urine were determined as in the previous experiments. The curve representing the iodine concentration is given in Diagram 4 which sets out, for comparison, the same subject's curves from the first experiment after the

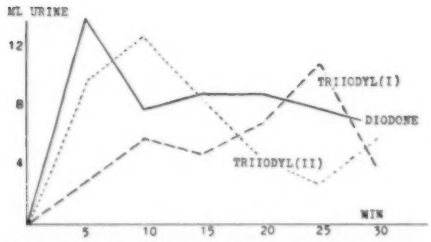

Diagram 5. Output of urine after administration of water and Triiodyl (II) compared with that caused by Diodone and Triiodyl (I) after usual dehydration.

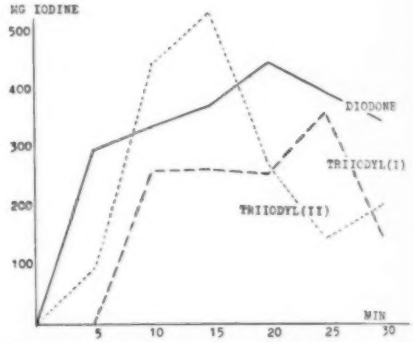

Diagram 6. Excretion of iodine after administration of water and Triiodyl (II) compared with excretion of iodine with Diodone and Triiodyl (I) after usual dehydration.

usual dehydration. This shows that the iodine concentration in the urine is approximately the same, whether Triiodyl is injected after dehydration or after the administration of water. The density of the urograms, therefore, ought to be equally good after the controlled administration of fluid. Diagram 5 sets out the curve representing the output of urine compared with the same subject's curves after dehydration. After the administration of water, the Triiodyl curve resembles the Diodone curve from the first experimental series, and this accords with the better filling of the calyces in the films. Lastly, in Diagram 6, the individual curves for this subject show how the increased output of urine after the administration of water results in a considerable increase of iodine excretion which is brought up to or above the level seen with Diodone.

Conclusion

The present study has established that with ordinary preparation of the patient, Diodone is preferable to Triiodyl for routine urography. (The question of nephrographic effect is left out of this discussion.)

Owing to the increased excretion of Triiodyl after the administration

of water, this medium may be applicable with advantage for patients who have not been prepared by dehydration before the investigation, that is in most acute cases, and possibly also for patients in whom such preparation is inadvisable or does not have the usual effect. This will be the subject of continued studies.

SUMMARY

The assessment of the effectiveness of urologic contrast media as hitherto from the film appearances has proved insufficient. The present study took the form of comparative urographies with Diodone and Triiodyl combined with chemical estimation of the iodine contents in the urine. Diodone proved superior for the purposes of routine urography, whereas Triiodyl presumably has its particular range of indication when patients cannot be prepared in the usual way or when the renal concentration is reduced.

ZUSAMMENFASSUNG

Die bisher übliche Beurteilung der Effektivität urologischer Kontrastmittel nach dem Aussehen der Röntgenbilder hat sich als unzureichend erwiesen. Die vorliegende Studie geschah in der Form vergleichender Urographien mit Diodon und Triiodyl, welche mit der chemischen Bestimmung des Jodgehaltes im Urin kombiniert wurden. Diodon war für die rutinmässige Urographie überlegen, während Triiodyl vermutlich besonders dann indiziert ist, wenn die Patienten nicht in üblicher Weise vorbereitet werden können oder wenn das Konzentrationsvermögen der Nieren herabgesetzt ist.

RÉSUMÉ

L'appréciation de l'efficacité des moyens de contraste urologiques, faite jusqu'à présent d'après l'aspect des films, s'est révélée insuffisante. La présente étude a consisté en urographies comparatives avec la Diodone et avec le Triiodyl, associées à une estimation chimique de la teneur en iode de l'urine. La Diodone s'est montrée supérieure pour l'urographie ordinaire, alors que le Triiodyl est probablement particulièrement indiqué chez les malades qui ne peuvent subir la préparation habituelle ou qui ont une concentration rénale réduite.

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FROM THE RESEARCH INSTITUTE OF NATIONAL DEFENCE, DEPT. 1,
SUNDBYBERG, SWEDEN

AN IMPROVED METHOD FOR MACROSCOPIC RADIOAUTOGRAPHY

by

Arne Nelson

In a chain reaction, uncontrolled as in the case of an atomic explosion, or controlled as in a reactor, some 90 radioactive isotopes are produced of which J^{131}, Sr^{89}, and Sr^{90} are regarded to be the most dangerous from the biologic viewpoint. The hydrogen bomb has furthermore increased the risk since the radioactive fallout may be dissipated over a world-wide area. The dangerous radioactive fallout from the hydrogen bomb of 1 March 1954, which had a TNT equivalent of 14 megaton was spread over an area of 18,000 km².

It would therefore seem to be of importance to investigate the metabolism of the radioactive isotopes of biologic significance.

The most important of the fission products is probably radioactive strontium which is chemically related to calcium. Bone consists principally of calcium phosphate, and for this reason radiostrontium like calcium is deposited in bone. Strontium 90 is considered to be the most dangerous of the strontium isotopes due to its long half life of 28 years. Because of its bone-seeking property it stays in the body for a long time. If the body content of radiostrontium exceeds a level 10 times the maximum permissible concentration, which is 1 microcurie for standard man, an increase in the number of bone tumours may be expected (LIBBY 1956).

For the investigation of the strontium metabolism, radioautography or measurement of radioactivity in dry-ash and wet-ash material, are generally used. Both these methods are cumbersome and time-consuming. There is accordingly the need for a simple macroscopic autoradiographic method, and such a method has been worked out at our institute.

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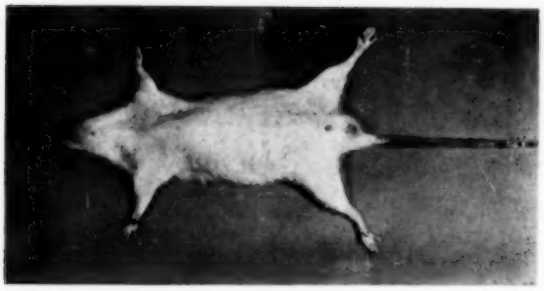

Fig. 1. Rat fixed to wooden plate and stretched by rubber bands.

Methods. 10 μc of Sr^{90} as $SrNO_3$ solution were injected intraperitoneally in rats. The animals were sacrificed, at intervals varying from 5 minutes to 128 days after injection.

The sacrificed rats were fixed with the back against a wooden plate and stretched by means of rubber bands, fastened to the incisors (Fig. 1). The plate with the rat was immediately put into a refrigerator at — 20° C and kept there for at least 10 days in order to obtain an equilibrium between Sr^{90} (half life 28 years) and Y^{90} (half life 65 hours); the latter may not have the same localization in living tissue as strontium. In the deep-frozen state the rat kept hard and very straight and was divided with a band-saw into two similar halves by a median cut running through the incisors, the skull, the vertebrae of the spine, and the tail.

The legs were removed from one of the halves which was employed for macroscopic autoradiography. The other half was used for microscopic autoradiography, microradiography, and other biophysic investigations.

For the macroscopic autoradiography a special pressure film-holder was constructed; this consisted of a metal plate in which holes and channels, communicating with a small tube at the wider end of the plate, were drilled (Figs. 2 and 3).

The macroscopic autoradiography was performed in the following manner: An industrial roentgen film, 10 × 40 cm, covered with a very thin sheet of cellophane for protection, was positioned on the surface of the plate. The rat was placed with the cut surface towards the cellophane. The film-holder with the rat was placed in a plastic bag which was affixed to the widened end of the plate by means of vaseline. The tube at the end-surface was connected to a water-jet pump and the bag evacuated by the channels in the plate. Due to the atmospheric pressure the specimen was uniformly and steadily pressed to the film, this being necessary in order to reduce the interspace between it and the sample (Fig. 4). During the exposure the whole apparatus was placed in the refrigerator in a light-tight box. The same type of film, exposure time (40 min), and processing conditions were used for all the examinations.

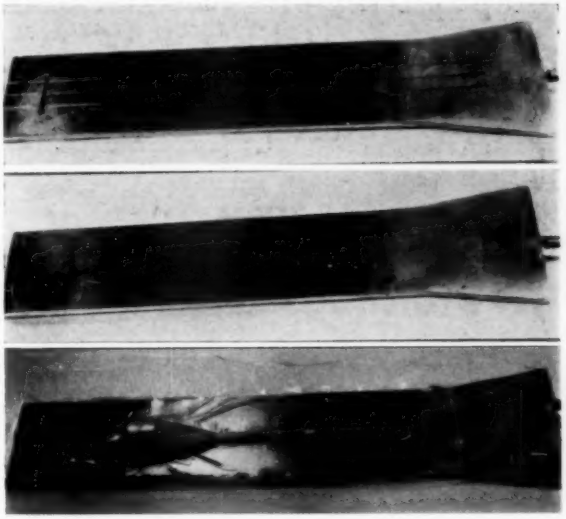

Fig. 2. Pressure film-holder from above.

Fig. 3. Pressure film-holder from below.

Fig. 4. Pressure film-holder in use.

From a series of macroscopic autoradiographic films (of which only four, Fig. 5, are reproduced in this article), from rats sacrificed 5 min to 128 days after an intraperitoneal injection of 10 μc Sr^{90}, it may be seen that the isotope is beginning to be distributed throughout the skeleton, with the exception of the tail, as early as after 5 min. The amount of isotope increases in the skeleton, including the tail, up to 6 hours, but there is very little decrease from 1 to 16 days. The different parts of the single bones are clearly seen, the metaphysis showing a much higher density than the diaphysis. After 32 and 64 days, the amount of isotope decreases. There is no appreciable difference between 64 and 128 days, indicating that some kind of equilibrium has been reached.

A very interesting fact is the increasing concentration of Sr^{90} in the incisors. In the films from the 6th hour to the 4th day the incisors show a very high density throughout their whole length. This high density area seems to follow the growth of the incisors moving towards the tips from 8 days to 32 days and disappearing after 64 days. The continuously growing incisors of the rodent are renewed in about one month.

From the macroscopic radiographic images obtained by the method described it is possible to obtain a rapid conception of the over-all distribution and retention of radioactive isotopes in the body of experimental animals.

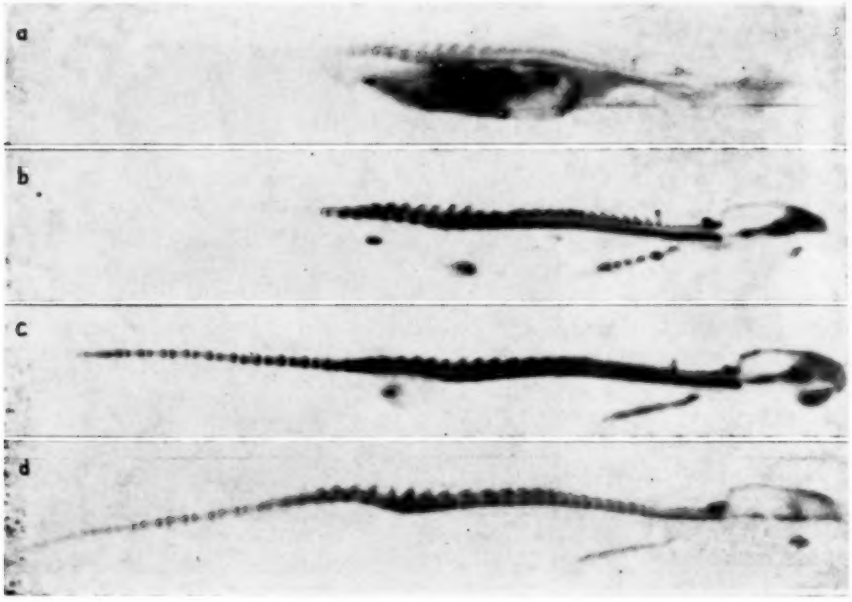

Fig. 5. Radioautographs of rats, a) 5 min b) 1 hour c) 5 days and d) 128 days after an intraperitoneal injection of 10 μc of Sr^{90}.

SUMMARY

A simple macroscopic autoradiographic method for the investigation of the distribution and retention of radioactive isotopes is described.

ZUSAMMENFASSUNG

Eine einfache makroskopische Autoradiographiemethode zur Untersuchung der Verteilung und der Retention von radioaktiven Isotopen wird beschrieben.

RÉSUMÉ

L'auteur décrit une méthode simple d'autoradiographie macroscopique pour l'étude de la distribution et de la fixation des isotopes radioactifs.

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FROM THE NORSK HYDRO INSTITUTE FOR CANCER RESEARCH, THE NORWEGIAN RADIUM HOSPITAL, OSLO, NORWAY

THE APPLICATION OF THE FERROUS-FERRIC SYSTEM FOR ROUTINE MEASUREMENTS

by

Johan Baarli and *Per Borge*

The chemical dosimeter in which the oxidation of ferrous ions to ferric ions in an aqueous solution of sulfuric acid by ionizing radiation is carried out, has been studied extensively (FRICKE and MORSE, FRICKE, HOCHANADEL and GHORMELY, WEISS et coll., LUZO et coll., SALDECK and ALLEN).

The yield, or G value, defined as the number of ferrous ions converted per 100 eV radiation energy given off to the solution has been experimentally found, and values lying between 16 (CORMACK et coll., WEISS et coll.) and 20 (CHAPIRO et coll.) are reported. Recent investigations of the geometric corrections which have to be applied when the yield is deduced from ionization chamber measurements in narrow geometry have been published (DYNE). Applying these corrections to the ionization chamber measurements, the agreement between the G values obtained by different workers seems to be closer. This value can now be regarded as well established for 200 kV roentgen rays, Co^{60} radiation and 23 MeV betatron radiation and is 15.9 ± 0.4 (WEISS et coll., LAZO et coll., SALDECK and ALLEN, CORMACK et coll.).

Experiments carried out show no significant difference in the yield for these radiation qualities, which means that variations in the ion density lying between the limit of 23 MeV betatron radiation and 200 keV roentgen rays have no detectable influence (CORMACK et coll.).

In the low energy region, however, the G value seems to be more dependent on the specific ion density. Experiments recently carried out (COTTIN and LEFORT), using 8 keV roentgen rays give a G value of 13.4, which indicates that the ionic yield decreases with the increasing ion density. It is important to take this into account if the dosimeter is to be employed in the low energy region.

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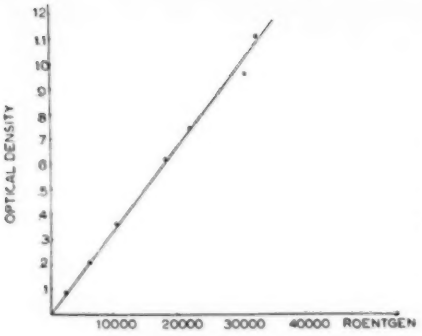

Diagram 1. Optical densities of a solution of ferric ammonium sulfate and 0.4 M H_2SO_4 measured for different wavelengths between 2,900 and 3,150 Å.

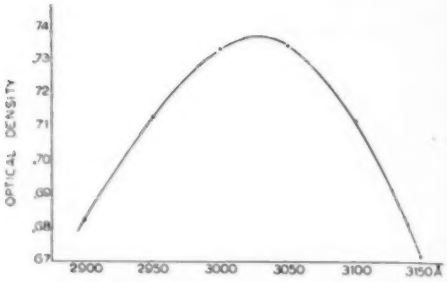

Diagram 2. The optical density of a 0.4 M H_2SO_4 solution as a function of the concentration of ferric ions added measured at 3,040 Å.

The dosimeter, however, is very suitable for 200 keV roentgen rays, Co^{60} radiation, and roentgen rays with higher energies where the G value can be assumed to be quite constant.

The investigations to be reported have been concerned with the practical problems in connection with the use of this dosimeter. Special attention has been paid to factors considered to be of importance in obtaining results which were reproducible.

The questions of interest concern the reliability of system and the precautions which must be taken in order to obtain a certain order of accuracy.

Sample preparations and measurements. Studies of the oxidation of ferrous ions to ferric ions by ionizing radiation have shown that this process is independent of the original ferrous ion concentration in the range 10^{-2} M to 4.10^{-5} M. (FRICKE and MORSE; FRICKE, FRICKE and HART). The experiments reported here made use of a concentration of 10^{-3} M Fe^{++}. The ferrous ions were added in the chemical form of Mohrs salt, $FeSO_4(NH_4)_2\ SO_4 6H_2O$, to a solution 0.4 M H_2SO_4. Triple distilled water stored in quartz bottles was always used. The distillation processes were carried out by adding 2 mg $KMnO_4$ and a few drops of KOH to each litre of water. The $KMnO_4$ was added in order to reduce organic dissolved impurities usually present in fresh water and the KOH in order to neutralize any acids present. The distillation equipment and test-tubes used as containers during the irradiation were all made of Pyrex glass; they were carefully cleaned with sulfuric acid, rinsed with distilled water, and dried before use.

The concentration of ferric ions formed by the radiation was measured by a Hoover Uvispec spectrophotometer in which the housing for the quartz cuvettes is separated from the lamp compartment. By this means the heat produced by the lamp has but slight influence on the temperature of the solution to be measured. In order that the temperature during a measurement might be controlled, the housing of the cuvettes was furnished with a thermometer.

The temperature difference between the irradiated solution and the cuvette housing usually observed was 4 to 5° C. Studies of the time needed to obtain thermal equilibrium after the solution was placed in the cuvettes were carried out by a thermocouple attached to the wall of the cuvette. As a result of this all readings were taken 10 min after the solutions were placed in the spectro-photometer. The procedure of measurement made use of unirradiated solutions as reference points.

An experiment was carried out to determine the wavelength for the selective absorption caused by the ferric ions. This was done by making a solution of ferric ammonium sulfate in 0.4 M H_2SO_4. Zero reading was taken on the 0.4 M H_2SO_4. The result of this investigation is shown in Diagram 1.

The curve reveals that the maximum absorption is obtained at 3,040 Å and that a small variation of this wavelength has no appreciable influence on the measurements. Varying the spalt width between 0.1 and 1 mm did not affect the measured optical density of the solution.

The spectro-photometer was calibrated by using known concentrations of Fe^{3+} ions in a 0.4 M H_2SO_4 solution. The known concentrations were obtained starting out from a standard solution of Fe^{3+} ions and diluting this with 0.4 M H_2SO_4. The standard solution was made up in two different ways. A known quantity of ferric ammonium sulphate, determined by weighing, was added to a 0.4 M H_2SO_4 aqueous solution. The other method of making up the standard solution was based on the determination of a known ferrous ion concentration by titration with permanganate and then carrying out a quantitative oxidation of the ferrous ions to ferric ions by H_2O_2. The calibration curve is shown in Diagram 2 and is referred to measurements at $24 \pm 0.3°$ C. The molar extinction of the ferric ions deduced from these measurements gave the value of 2,206.

An experiment was also carried out to determine the influence of temperature on the optical density of the ferric ions measured. A thermoelement was attached to the quartz cuvette. The temperature was slowly increased and the extinction measured. The results of the measurements for 3 different concentrations of H_2SO_4 are shown in Diagram 3.

These indicate that the molar extinction of the ferric ions increases with a raised temperature by 21 units per degree at 24° C for a 0.4 M H_2SO_4 solution.

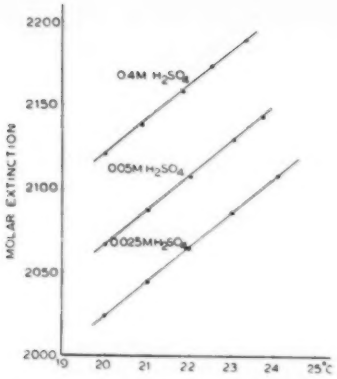

Diagram 3. The variation with temperature of the molar extinction of ferric ions using three different concentrations of sulfuric acid.

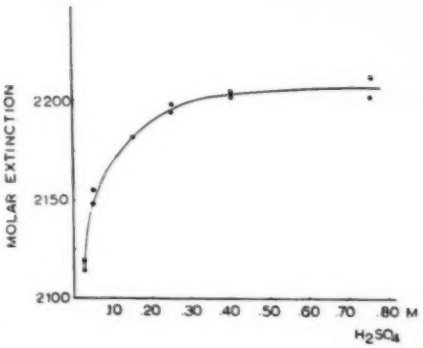

Diagram 4. The variation of the molar extinction of the ferric ions with the concentration of sulfuric acid.

By changing the molarity of the solvent for the ferric ions, a variation in the molar extinction of these ions is observed. This relationship is shown in Diagram 4. It is easily seen that above a concentration of 0.4 M H_2SO_4, the molar extinction is quite constant and a small change in the molarity has no effect on the molar extinction measured.

The influence of the presence of ferrous ions on the measured concentration of ferric ions in the solution was investigated by making up different concentrations of ferrous ions and keeping the same concentration of ferric ions. The results of these measurements showed that the presence of ferrous ions did not have any influence on the measured concentration of ferric ions.

These measurements were carried out using a concentration of 315 μ M Fe^{3+}, the solution containing 0,325, and 475 μ M Fe^{2+}.

These preliminary studies show that for a dosimeter in which ferrous ions are oxidized to ferric ions, a solution containing 10^{-3} M Fe^{3+} ions in an 0.4 M H_2SO_4 aqueous solution made up of triple distilled water and using p. a. chemicals should be employed. The temperature should be recorded during the measurement of the ferric concentration.

It can also be said that smaller variations caused by uncertainty in the concentration of sulfuric acid and in the concentrations of ferrous ions may be disregarded. The influence on the extinction measured by the presence of ferrous ions may also be neglected.

Dose measurement by the ferrous-ferric system. The ferrous-ferric system was used as a dosimeter by irradiating 2 ml of the solution in

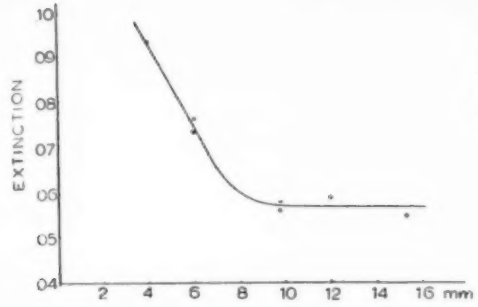

Diagram 5. Concentration (optical density) of ferric ions, produced by the same roentgen dose, as a function of the internal diameter of the irradiated test tubes.

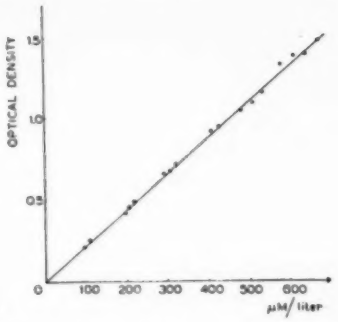

Diagram 6. The optical density as a function of the total roentgen dose given to the solution.

Pyrex test tubes. The irradiation was carried out with a 175 keV roentgen unit with a 0.5 mm Cu filter, and a Victoreen type thimble chamber as monitor in fixed geometry. Since the effective atomic number of Pyrex glass is considerably greater than that of water, it is to be expected that the wall thickness and diameter of the test tubes will influence the measured dose. The wall thickness of 1 mm was chosen in order to obtain electronic equilibrium. An investigation was then carried out on the possible effect of using test tubes of different internal diameters the wall thickness being kept at 1 mm. Diagram 5 shows the result and indicates that the test tubes with internal diameters of 10 mm or more to be practical as irradiating tubes; all irradiations were carried out with tubes of 10 mm internal diameter and 1 mm wall thickness.

The reproducibility was checked by carrying out 22 separate runs, the solution receiving between 1,000 and 1,200 r. The dose rate was of the order of 100 r/min, and temperature corrections were applied to each measurement. The mean deviation for these runs was 6 %. A similar series was carried out with doses of between 5,000 and 6,000 r to the solution; the deviation was then 4 %. The somewhat greater deviation at lower doses may be explained by the uncertainty in the measurements of the very low concentration of ferric ions (KOLTHOFF & SANDELL).

The dose calibration of the system was carried out by means of two 500 r Sievert condensor chambers placed in the test tube. These chambers were previously calibrated by a Victoreen type thimble chamber.

The linear relationship between dose and yield was measured by a series of runs with doses of up to 32,000 r to the solution. The results of these measurements are shown in Diagram 6.

The yield deduced from these measurements is 15.7 ± 0.8 μ M Fe^{+3} per litre per 1,000 r, which is in close agreement with the work of others.

Conclusion. From the experimental studies described it is concluded that dose measurements by means of the ferrous-ferric system, adequate precautions being taken in making up the solutions, will give reproducibility better than 6 % when temperature corrections are applied and doses of 1,000 r or more are measured.

SUMMARY

A series of studies of the oxidation of ferrous ions to ferric ions in an aqueous solution of sulfuric acid irradiated with roentgen rays have been carried out in order to apply this system for routine dose measurements. Quantitative information about the variation in the measurements caused by the temperature, the concentration of sulfuric acid, and total dose has been investigated.

ZUSAMMENFASSUNG

Eine Reihe von Untersuchungen über die Oxydation von Ferrojonen zu Ferrijonen in wässeriger röntgenbestrahlter Schwefelsäurelösung sind ausgeführt worden, um dieses System für rutinmässige Dosismessungen zu benutzen. Quantitative Befunde über die durch die Temperatur, die Schwefelsäurekonzentration und die totale Strahlendosis hervorgerufene Variation der Messungsresultate sind sorgfältig untersucht worden.

RÉSUMÉ

Les auteurs ont fait une série d'études de l'oxydation d'ions ferreux en ions ferriques dans une solution aqueuse d'acide sulfurique irradiée par des rayons roentgen, en vue d'appliquer cette méthode à la dosimétrie pratique. Ils ont étudié quantitativement l'influence de la température, de la concentration de l'acide sulfurique et de la dose totale sur le résultat des mesures.

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FROM THE RADIUM CENTRE FOR JUTLAND, AARHUS, AND THE DEPARTMENT OF RADIOTHERAPY, CENTRAL HOSPITAL, RANDERS, DENMARK.

CLINICAL EXPERIENCE WITH CB 1348 (NN-DI(2-CHLOROETHYL,-P-AMINOPHENYLBUTYRIC ACID) IN THE TREATMENT OF CHRONIC LYMPHATIC LEUKAEMIA

by

Poul Bjerre Hansen

The results of a series of experimental and clinical investigations on the therapeutic efficacy of a new nitrogen-mustard derivative, CB 1348 = NN-di(2-chloroethyl)-p-aminophenylbutyric acid, were reported by HADDOW at a conference in London in November 1953.

This new agent, which is a member of the carboxylic-acid derivatives in the nitrogen-mustard series, has been produced and studied at the Chester Beatty Research Institute, London, just as many other different nitrogen-mustard derivatives, during the last 8 to 9 years. The purpose of these investigations has been partly to endeavour to produce compounds with the greatest possible therapeutic efficacy and partly to study the mode of action of these growth-inhibiting agents and the relation between their chemical structure and reactivity and their biologic activity.

Like all the other derivatives, CB 1348 was subjected to various studies in tumour-bearing animals. It proved to have a very strong inhibitory effect on the Walker tumour in the rat, while its effect on the Krebs-II ascites tumour and various transplantable leukaemias in the

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mouse was less pronounced. Just as all other nitrogen-mustard derivatives and radiomimetic substances as a whole, CB 1348 induces fragmentation of chromosomes and other mitotic abnormalities. Blood examinations (in rats and mice) showed that a single dose of 12 to 15 mg/kg body weight induced a rapid and marked reduction in the number of lymphocytes and, a little later, a moderate increase in the granulocyte count. These changes were completely reversible. Doses of this size did not exert any appreciable effect on the red cell count. This fairly pronounced depressive action on the 'lymphocytes' presents an interesting contrast to the relatively specific inhibitory activity of Myleran on granulopoiesis. As emphasized by ELSON, who has studied these aspects in detail, it is noteworthy that a combination of the effects of these two agents would induce changes in the blood picture similar to those seen after roentgen irradiation.

On the basis of the aforementioned experimental investigations, CB 1348 was selected for a clinical trial (GALTON and TILL), principally in cases of malignant disease arising from the lymphatic system, since the agent, as already mentioned, seemed to exert an inhibitory action on cells and tissue of this type. Up to November 1953, a total of 28 patients had been treated with CB 1348, including nine with lymphosarcomatosis, two with chronic lymphatic leukaemia, nine with Hodgkin's disease, and four with reticulum cell sarcomatosis. Relatively long-continued improvement was obtained in two of the patients with chronic lymphatic leukaemia and in three of those with lymphosarcomatosis — all three had 'follicular lymphosarcomata' — and, finally, in one with reticulum cell sarcomatosis. Many of the other patients showed good immediate response, but the effect was short-lived. A few patients whose disease was in the terminal stage and three with acute lymphatic leukaemia, acute myeloid leukaemia, and multiple myeloma, respectively, derived no real benefit from the treatment. In one patient with mycosis fungoides a fairly pronounced but short-lived improvement was seen. CB 1348 was given orally in tablets of 2 mg, the average daily dose being 0.2 mg/kg body weight during the first 2 to 3 weeks and 0.1 mg/kg during the following weeks. In most cases, the treatment extended over a period of 4 to 8 weeks. No special side-effects were observed but, as by the use of most other nitrogen-mustard derivatives, a certain depressive action on the bone marrow was noted; however, this reaction was moderate in degree and never of an irreversible character.

At the Radium Centre for Jutland, Aarhus, we have had an opportunity to treat a series of patients with CB 1348. The work has been carried out in close contact with the medical staff of the Chester Beatty Research Institute who generously supplied us with the drug.

We principally selected patients with chronic lymphatic leukaemia

for this treatment; from January 1954 to March 1955 a total of 13 such patients were treated with CB 1348 in Aarhus. During the same period another two patients with chronic lymphatic leukaemia and two with Hodgkin's disease were treated in the Central Hospital, Randers.

Since, as was also emphasized by the British investigators, the bone-marrow function of patients with chronic lymphatic leukaemia is particularly sensitive to nitrogen-mustard compounds, we decided to use a dose of about one half of that used by GALTON and TILL. As a rule, the daily dose was 6 mg — 1 tablet of 2 mg being given three times daily — during the first few weeks and then 4 or, in most cases, 2 mg daily during the following weeks. The average total dose given, or at least aimed at, was 150 to 200 mg in 6 to 8 weeks. Protraction of the treatment aiming at a maintenance therapy was given a trial in some cases, partly by temporary withdrawal of the drug and partly by further reduction of the dose to 6 or 4 mg weekly. However, maintenance therapy was successfully achieved only in one case in which the treatment extended over about 12 months (Case 1). A relatively protracted course of treatment over a period of about 3 months was given in six cases, and treatment of the same intensity but of shorter duration (1 to 2 months) was accomplished in three cases. In the remaining five patients with chronic lymphatic leukaemia the treatment had to be interrupted for various reasons and could not be regarded as adequate. The two patients with Hodgkin's disease were both treated for periods of 3 to 4 months.

The duration of treatment, total dose and various other data concerning the 17 patients are shown in Table 1 together with a survey of the value of the therapy in the individual cases up to June 1956, *i.e.* after an observation period of at least 14 months.

Three of the 15 patients with chronic lymphatic leukaemia were women, and both these women and the 12 men in the group were of advanced age. The average age was 64.5 years. The duration of disease ranged from 1 to 6 years in 10 of the patients, who had previously been treated in different ways, principally with roentgen irradiation, other nitrogen-mustard derivatives and, in some cases, with triethylene melamine. In the remaining five patients the duration of disease was only a few months.

The two patients with Hodgkin's disease had both been ill for 5 to 6 years. In both cases the disease was in the terminal phase, and the treatment with CB 1348 was only tentative because they had become 'refractory' to all other forms of therapy. A good palliative effect was obtained; pruritus and fever subsided, and some transitory improvement in the general condition occurred. The disease as such progressed steadily, and both these patients died a few months later.

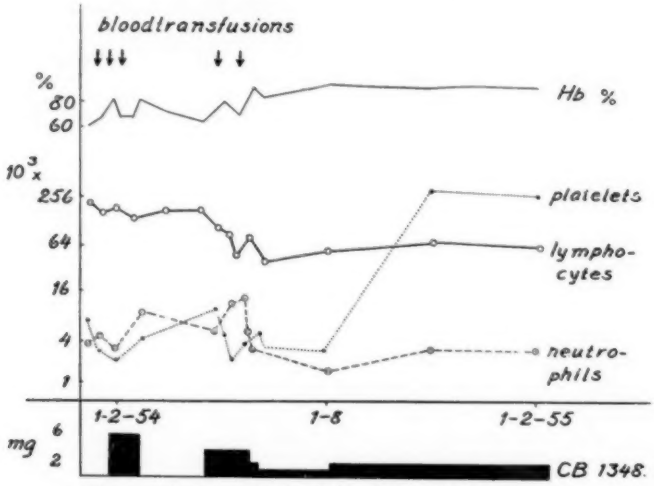
As shown in Table 1, four of the 15 patients with chronic lymphatic

Table 1. Data of treatment with CB 1348

Case	Sex	Age (yrs)	Duration of disease	Previous treatment	Duration of CB 1348 therapy	Total dose (mg)	Initial improvement	Duration of improvement		Therapeutic value		
								Short-lived	More than 3 ms	Significant	Dubious	None
Chronic lymphatic leukaemia												
Group A												
1	F	74	6 yrs	+	13 ms	700	+		+	+		
2	M	66	12 ms	+	4 »	300	+		+	+		
3	M	78	2 ms	—	3 »	280	+	+			+	
4	M	64	2 yrs	+	3 »	250	+	+			+	
5	M	51	3 ms	—	3 »	250	+		+	+		
6	M	69	2½ yrs	+	3 »	250	+		+	+		
7	M	63	12 ms	+	3 »	200	+	+			+	
Group B												
8	F	55	2½ yrs	+	2 »	180	+	+			+	
9	M	61	2 ms	—	1 m	150	—					+
10	M	62	4 ms	—	1 »	100	+	+			+	
Group C												
11	F	68	12 ms	+	3 wks	80	—					+
12	M	59	3 yrs	+	3 »	50	(+)					+
13	M	65	18 ms	+	2 »	30	—					+
14	M	67	6 ms	—	2 »	80	+	+			+	
15	M	65	3 yrs	+	2 »	90	—					+
Hodgkin's disease												
16	F	47	5 yrs	+	3 ms	330	+	+				+
17	M	33	6 yrs	+	4 »	300	+	+				+

leukaemia derived real benefit from the treatment. Their general condition and subjective well-being showed considerable improvement and the haematologic status approached normal, *i. e.* the haemoglobin level increased, occasionally supported by blood transfusions, and the lymphocyte count decreased. In another six patients the immediate effect was relatively good but so short-lived that the therapeutic significance must be described as dubious. In five cases the therapy seemed to be without any beneficial effect at all. However, four of these belonged to the group of patients who must be regarded as having received inadequate treatment, but it must yet be pointed out that the fifth of the patients (Case 14) within this group (Group C) obtained some benefit in the course of 2 weeks on a total dose of only 80 mg, whereas the therapy was absolutely ineffective in a patient (Case 9) from group B in spite of a total dose of 180 mg given within 3 to 4 weeks. Thus, there is no absolute parallelism between the results obtained and the duration and intensity of the treatment. The results do not seem to show any difference between previously

treated cases of long duration and untreated, 'fresh' cases. In two cases, CB 1348 induced a definite, although short-lived, improvement in patients who no longer responded to other treatment. In two patients (Cases 13 and 15), the disease was in the terminal phase; both died during the initial stage of treatment, but the therapy is not believed to have contributed to the fatal outcome in these two cases. Of the 15 patients, another five (Cases 4, 7, 8, 14 and 6) died 2, 6, 10, 12 and 17 months, respectively, after the conclusion of treatment. The remaining eight patients are still alive, but have all subsequently been given other forms of therapy. The remissions which were obtained by CB 1348 therapy were thus relatively short, usually from 2 to 4 months, and only in two patients (Cases 5 and 1) did the remissions persist for 10 to 12 months.



The remissions obtained by CB 1348 in Case 1.

The influence of the drug on the bone-marrow function is illustrated by the curves for the blood-cell counts in Case 1 both with regard to the aforementioned reduction in the lymphocyte count and the transitory increase in the granulocyte count. The curves also show that CB 1348 had an excellent stabilizing effect on the blood picture, and that judging from this a sort of maintenance therapy was obtained in this case. A pronounced depression of the bone-marrow function with development of aplastic anaemia, such as is encountered after the treatment with several other nitrogen-mustard derivatives, was not observed. However, a moderate depression was seen in a few cases, but it disappeared rapidly following the cessation of therapy or merely a reduction of the dose, or

when one or two blood transfusions were given. It seems as if the platelet production is the function which is most sensitive to CB 1348. Accordingly, the haematologic status of the patients should be closely followed during treatment; in this connexion it must be recalled that the dosage used in this series was very small in order to ensure that side-effects, particularly the undesired affection of the bone marrow, should not be too pronounced. Incidentally, only mild side-reactions were noted. A few patients complained of slight nausea, but this did not affect their general condition or influence the treatment in any of the cases.

Conclusions

It must therefore be concluded that we have here a new anti-neoplastic chemotherapeutic agent, which seems to exert a relatively specific action on malignant conditions arising in the lymphatic tissue. The clinical value of CB 1348 is, perhaps, rather limited, but in suitable cases it should be possible to obtain long-continued improvement through a cautious, protracted course of treatment, as was seen in a few of the patients of the present series. In addition, this agent is of considerable theoretic and experimental interest.

Addendum (February 1957)

Since this paper was submitted for publication a report on the treatment of chronic lymphocytic leukemia and certain lymphomas with CB 1348 has been published by Ultmann, Hyman & Gellhorn (J. A. M. A. 162 (1956), 178. Here the compound is called CHLORAMBUCIL. 30 patients were treated (18 cases of chr. lymph. leukemia, 6 of Hodgkin's disease and 6 lymphomas). The results reported are very similar to ours.

SUMMARY

The results in a series of 17 cases treated with CB 1348 are reported and discussed. The clinical value is rather limited but in a few of the cases in the present series an improvement was obtained through a cautious protracted course of treatment.

ZUSAMMENFASSUNG

Die Ergebnisse einer Serie von 17 Fällen, welche mit CB 1348 behandelt worden sind, werden berichtet und diskutiert. Der klinische Wert ist ziemlich begrenzt; jedoch wurde in einigen wenigen Fällen der vorliegenden Serie durch vorsichtige protrahierte Behandlung eine Verbesserung erreicht.

RÉSUMÉ

L'auteur présente et analyse les résultats obtenus sur une série de 17 cas traités par le CB 1348. Son intéret clinique est assez limité, mais, dans quelques-uns des cas de cette série, un traitement prudent et prolongé a donné une amélioration.

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FROM RADIUMHEMMET (DIRECTOR: PROF. SVEN HULTBERG) AND THE INSTITUTE OF RADIOPHYSICS (DIRECTOR: PROF. ROLF SIEVERT), KAROLINSKA SJUKHUSET, STOCKHOLM, SWEDEN

A SCANNING APPARATUS FOR THE LOCALIZATION OF GAMMA EMITTING ISOTOPES IN VIVO

by

Lars Jonsson, Lars-Gunnar Larsson and *Inger Ragnhult*

Gamma-emitting radioisotopes may be administered internally for physiologic studies, or for diagnostic, or therapeutic purposes. The penetrative character of gamma rays makes it possible to study the distribution of the isotope *in vivo*. Due to the difficulty to collimate gamma rays, and due to the variable distance of the radiation source from the measuring instrument, the absorption of radiation in the tissues, and the overlapping of different organs within the body, such studies must always be relatively rough. Nevertheless, a careful study *in vivo* of the distribution of a radioisotope, in a special part of the body or in the total body, often gives information of considerable medical interest.

Several methods have been tried to arrive at a 'picture' of the radioactivity in the body. The gamma rays from the body may be collimated either with a pin-hole camera (4, 11, 26, 27), or a thick lead grid (20, 21), and caused to blacken a photographic film. The image may be intensified by placing scintillation crystals next to the film (20, 21, 26). All these purely photographic methods are, however, too insensitive for practical use in connection with tracer doses of radioactive isotopes. They are nevertheless of considerable interest and may be further improved by the use of electronic image amplification (21, 26).

In practice, the instruments most used have been directional G. M. or scintillation counters. The strongly collimated counter may be displaced manually, point by point, over the part of the body examined

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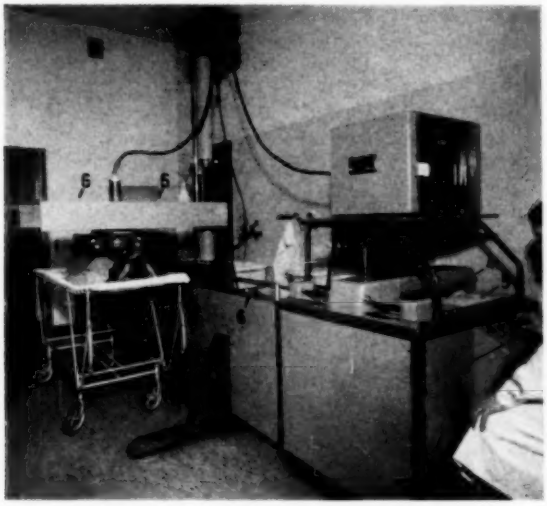


Fig. 1. The scintillograph. 1. Detector head. 2. Control panel for manual setting of detector head. 3. Main control panel for movements and recording. 4. Mechanical printer. 5. Recording paper. 6. Limiting switches for movements of detector head during scanning.

and the counting rates at each point registered and compared. This method is time-consuming and inconvenient which often means that the demand for detail is minimized. Motor-driven scanning devices, in which the detector systematically transverses the object and where the counts (or a certain fraction of them) are recorded as spots on a paper, or a photographic film, have therefore many advantages (2, 3, 5—9, 13, 15, 16, 19, 22—24, 29—31). Most apparatus have been designed for scintillation counters, which have a much greater sensitivity for gamma radiation than G. M. counters. The automatic device has been called a 'scintigraph', 'scintillograph', 'scintiscanner' or 'gammagraph' and the image obtained a 'scintigram', 'scintillogram' or 'gammagram'.

The scintillograph to be described has been designed at Radiumhemmet and is manufactured by LKB-Produkter Fabriksaktiebolag, Stockholm. In particular, it was constructed to meet the clinical demands, which appeared during radioiodine studies of the thyroid gland and thyroid carcinomas, and in connection with the intravenous, intracavitary, and interstitial use of radioactive colloids; but it is of course also useful in many other situations when the *in vivo* scanning of radioisotopes is needed. Good mechanical reliability, great variability, and simple management are desirable characteristics of the apparatus. Its use is exemplified by a few scintillograms.

The apparatus. The scintillograph is illustrated in Fig. 1. The detector head (1) is driven from a single motor through a gearbox in which electromagnetic clutches control the different movements. The motor and gearbox are relay-operated through limiting switches (6).

The apparatus may be employed in three principle ways:

1) Measurements with a stationary detector head.

2) Recording of s. c. profile diagrams. This type of measurement is a simple and reproducible method of studying the distribution of radioiodine in patients with functioning thyroid carcinoma metastases (28). For this purpose, the detector head is provided with a slot-formed collimator and moves over the body in only one direction with a speed of 8.75 or 17.5 cm per minute.

3) Scanning. The detector head provided with a narrow cylindric collimator transverses the object in a series of parallel lines and the rectangular area examined may be varied up to 56×170 cm². The radial scan used in certain apparatus described may simplify the mechanical device (13, 15, 23, 24, 29) but gives more distorsion of the picture and is definitely unsuitable for scanning of large areas. The distance between two adjacent lines (spacing) is in our scintillograph standardized to 0.3, 0.5, 1.0, 1.5, 2.5 or 5.0 cm. Two different speeds can be selected: 14.0 and 38.5 cm per minute.

Recording system. At measurements with the stationary detector head the counts may be read on the scaler or on a counting rate meter. For continuous registration of counting rates, which are rapidly changing with time, and of profile diagrams, the rate meter is combined with a pen recorder. The counting rate meter is also convenient during preliminary measurements over the examined area for the purpose of selecting suitable collimator, speed, and scaling factor for scanning.

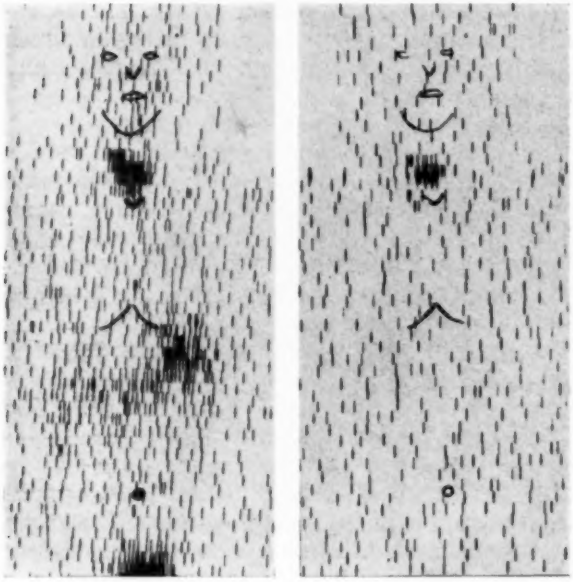
For the registration of the counts during the scanning procedure several methods have been described:

Direct mechanical printing on paper (2, 3, 6, 8, 12).

Electric recording on Teledeltos paper (29, 19).

Photographic recording of light impulses obtained from an electric spot light (13, 15, 16, 9) or a cathode ray tube (23, 24). The impulses from the latter may also be stored in a storage tube and the spots built up to a picture persisting on a television screen (22).

Methods to increase the contrast in the scintillogram by arrangements in the recording system have also been tried. A simple but rather artificial way is to suppress registration when the counting rate is below a certain level, or to increase the scanning speed over such areas (12). On photographic recording it is possible to make both the frequency and the intensity of the light impulses dependent on the counting rates (9).



a b

Fig. 2. Total body scintillograms two and six days after administration of 3 mC I^{131} in patient with myxedema after previous thyroidectomy and I^{131} ablation. Total uptake in thyroid was less than 1 % of dose administered. After two days (a) highest counting rates are localized to thyroid, urinary bladder, and stomach (due to gastric secretion of radioiodide). After six days (b) when the radioiodide concentration in body has further decreased, the urinary bladder and stomach are no longer visualized. (Spacing 1.5 cm, speed 38.5 cm/min. cylindric collimator of 1.2 cm diam., 364 keV.)

The advantages of photographic recording are the absence of mechanical inertia, and the fact that overlapping spots increase the blackening of the film and thus are further useful for the contrast in the scintillogram. The photographic recording includes probably the best possibilities for future development, especially if combined with methods for contrast improvement (9). At present, however, the limitations of the scintillographic technique are more dependent on the collimator system and on unavoidable anatomic and biologic sources of error than on the recording system, regardless of which of the above-described methods is used.

For the recording from this scintillograph we have chosen simple mechanical printing on paper, which has several practical advantages. The scintillogram may be watched during its production, which permits correction of the positioning or scaling factor at an early stage. The scintillogram is ready for evaluation immediately after the scanning without time-consuming photographic processing. Several copies may also be printed simultaneously. The main disadvantage of the method is the inertia in the mechanical printing system.

The system consists of an electromagnetically driven punch (Fig. 1: 4), and a drawing table between which a typewriter tape and a sandwich of recording papers and carbon papers are placed. The resolv-

Fig. 3. Scintillogram recorded in scale 1 : 5 after injection of 35 mC Au^{198} in each parametrium. (Courtesy of Dr. H.-L. KOTTMEIER.) (Spacing 1.5 cm, speed 38.5 cm/min. cylindric collimator of 0.6 cm diam., channel corresponding to 411 keV.)

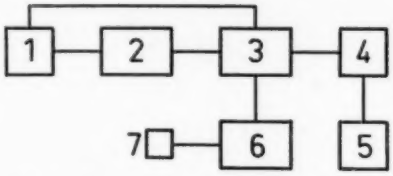


Fig. 4. Block diagram of detector and recording systems. 1. Scintillation detector 2. Pulse height analyzer. 3. Scaler. 4. Counting rate meter. 5. Pen recorder. 6. Printing circuit. 7. Printer.

ing time of the mechanical printing system for the recording of two consecutive impulses is about 0.03 sec. From the scaler, the pulses are taken with a dividing factor of 1, 10, 100, or 1,000 to the printing circuit. The latter consists of a pulse former and two stages 'scale of two' together with a driver stage for the printer. By this method scaling factors of 2, 4, 10, 20 . . . 1,000 may be selected. The scintillogram may be obtained in scale 1 : 1 or 1 : 5. The maximal area that can be scanned in scale 1 : 1 is 42 × 60 cm^2 and, in scale 1 : 5, 56 × 170 cm^2. The latter is preferable when large areas are examined (Figs. 2 and 3) and permits even total body scintillograms of convenient size.

Detector system. The detector and recording systems are schematically illustrated in Fig. 4. The scintillation crystal consists of a cylindric NaI (T1) crystal with a diameter of 1.5 and a height of 1.0 inches. The crystal is attached to a photomultiplier tube (DUMONT 6292). After amplification in a linear amplifier, the pulses from the photomultiplier are fed to a pulse height discriminator which permits pulses of desired height to pass to the scaler.

The use of a pulse height analyzer has several advantages (3, 14). A suitable photopeak from the measured isotope may be selected and the number of background counts greatly reduced, which increases the sensitivity. Scattered radiation from the tissue or from the collimator system can be largely kept out from registration, which reduces one error in the *in vivo* measurements. The improvement of the scintillogram obtained by measuring a suitable photopeak instead of the whole spectrum is illustrated in Fig. 5. Two radioisotopes with separated photopeaks, simultaneously present in the body, may also be measured more

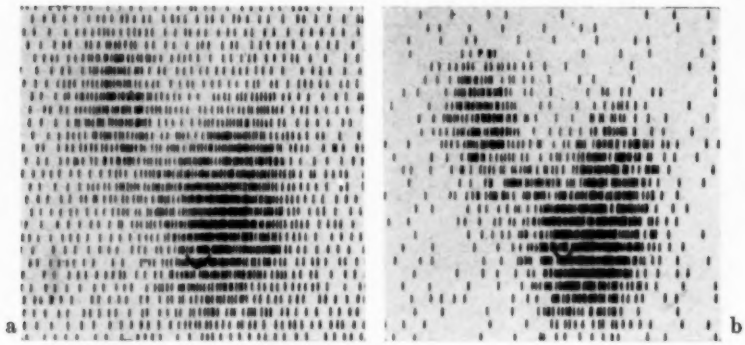
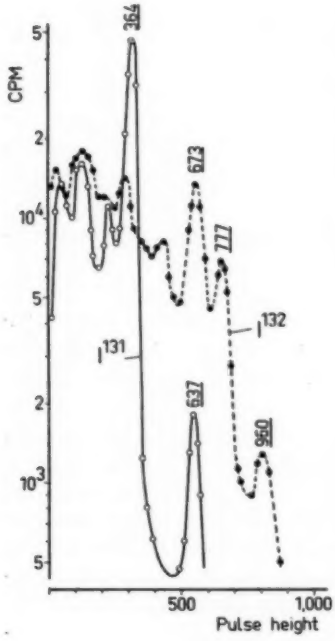


Fig. 5. Toxic nodular goiter scanned after a tracer dose of 0.06 mC I^{131}; uptake about 45 %. a) Scintillogram measured with open channel and, b) with a channel corresponding to the 364 keV photopeak of I^{131}. Spacing, speed, and collimator are the same in both; scaling factor is adjusted to obtain about same density of spots over thyroid in both. Observe improvement obtained in (b). (The jugular fossa is in these and in the following thyroid scintillograms marked with a V.)



or less independently. After a therapeutic dose of I^{131}, physiologic radioiodine studies with a conventional technique may for a long time be impossible due to the large amount of radioactivity remaining in the thyroid. I^{132} may, however, by the use of a pulse height analyzer, be measured independently of I^{131}; the principle for distinguishing these two isotopes is illustrated in Diagram 1 and Fig. 6.

Diagram 1. Pulse height spectrum of radiation from extended source of I^{131} and I^{132} respectively. Source is placed in a water phantom and the scintillation collimated with a cylindric lead collimator, 1.2 cm wide, in order to imitate conditions during *in vivo* directional measurements. The high energy photopeaks of I^{132} makes it possible to measure this isotope, independently of I^{131} in a mixture, by use of a single discriminator technique.

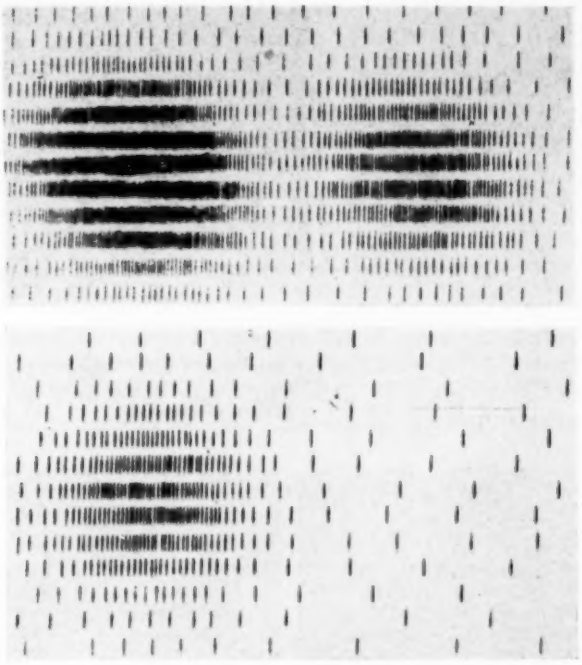


Fig. 6. Two spherical bottles, the left containing I^{132} and the right I^{131}, are scanned. In the *upper* scintillogram, only pulses corresponding to photon energies below 250 keV are discriminated and both bottles are visualized. In the *lower*, pulses corresponding to photon energies below 700 keV are discriminated and only the bottle containing I^{132} is visualized.

Lead shield and collimators. The lead shield consists of two parts (Fig. 1: 1), one fixed part and one interchangeable part containing the collimator system. The crystal is, with the exception of the collimator opening, protected in all directions with at least 5 cm lead. For our actual clinical need, three types of collimators have been designed. For measurements with the stationary detector head (for instance 'thyroid uptake of radioiodine') conical collimators with an opening angle of 12° and 34° respectively and a length of 8.5 cm along the central axis are used. For profile measurements a collimator with a 1.0 cm wide slot is used, which provides good collimation along one direction and no collimation along the perpendicular direction; the length of this collimator is also 8.5 cm along the central axis. For directional measurements and scanning, cylindric collimators with a diameter of 6, 12, and 24 mm are used;

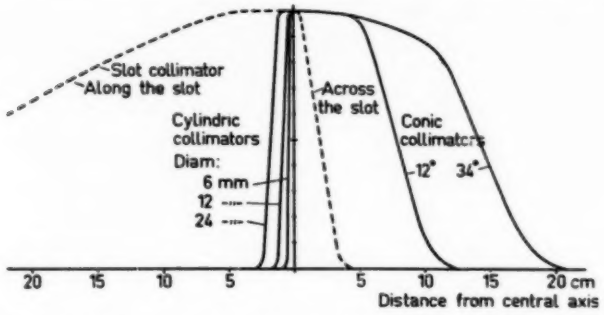
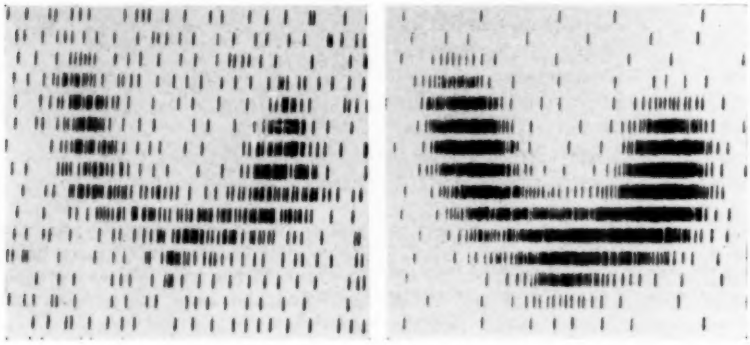


Diagram 2. Isosensitivity curves for different collimators, representing relative counting rates obtained when a point source of I^{131} is moved from the central axis. Crystal-source distance along central axis is 30 cm for the conic collimators and the slot-collimator and 15 cm for the cylindric collimators.



a b

Fig. 7. Scintillograms of an excised thyroid gland containing about 0.09 mC I^{131}; a) measured with a collimator of 0.6 cm diam., and b) with a 1.2 cm collimator. The latter looks better as a picture due to the improved relation between thyroid and background counts but gives a less true picture of the form and size of the gland than (a). Both were measured with an open channel.

the distance from the crystal surface to the collimator opening is 11 cm. The geometry of the collimators is illustrated in Diagram 2. The influence of different collimators on the scintillogram is exemplified in Fig. 7.

Control panels. The controls of the movements and the recording are confined to two panels. One is placed at the end of the console for the detector head and includes switches for the manual setting of the latter (Fig. 1: 2). The main panel (Fig. 1: 3) is situated on the recording table

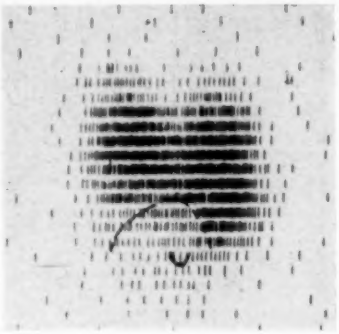


Fig. 8. Woman with hyperthyroidism and a palpable nodule in right lower part of thyroid. Tracer test with I^{131} (0.15 mC) revealed 24 hours uptake of 64 %. Scintillogram showed a generally enlarged gland with diffuse uptake with exception of the palpable nodule which took up very little or no radioiodine.

and contains the following switches: power switch, main switches for the three principle functions, start and stop switches for profile measurements, and scanning and selectors for speed, spacing, scaling factor and scale.

(If not otherwise mentioned, the following factors were used for the scintillograms published in this article: Scale 1 : 1; spacing 0.5 cm; speed 14.0 cm per minute; cylindric collimator of 1.2 cm diameter; channel in pulse height analyzer corresponding to the 364 keV photo-peak of I^{131}.)

Discussion of medical use

The scintillographic technique has at present its greatest practical importance in connection with radioiodine studies of the thyroid gland (2, 3, 6, 8, 15, a. o.). The localization and the shape of the active gland can be studied and its size determined (1), this latter being of special importance for the dosimetry in radioiodine therapy. The function of nodules within the gland may be studied (Fig. 8), and ectopic thyroid tissue, as lingual, prelaryngeal, and intrathoracic goiters (Fig. 9), may be diagnosed. Minor remnants of thyroid tissue after thyroidectomy or I^{131} ablation, not possible to diagnose by conventional uptake studies, and easily overlooked during manual directional measurements, may be localized and mapped out (Fig. 10). Metastases from functioning thyroid carcinomas may be diagnosed and studied. For the last purpose, a scintillograph which can scan large areas of the body is especially useful.

The localization of radioisotopes *in vivo* is of value also in several other situations. The distribution of a radioactive colloid can be studied after intrapleural or intraperitoneal injections for therapeutic purposes. The same is true after interstitial injections, where both the local distribution (fig. 4) and the uptake in the lymph nodes (cf. 17, 18) is of interest. The liver may be mapped out after the intravenous injection

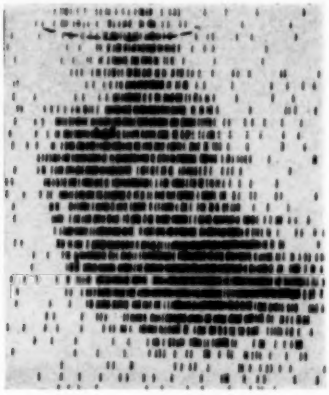


Fig. 9. Large, intrathoracic goiter in patient previously operated upon for nodular goiter. Roentgenography suggested a malignant lymphoma with hilar and mediastinal deposits. Scintillography, however, revealed a large intrathoracic goiter which was confirmed by aspiration biopsy. Tracer test performed with 0.15 mC I^{131}; uptake in the goiter about 45 %.

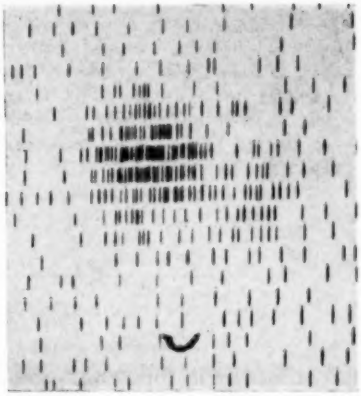


Fig. 10. Woman with myxedema after previous thyroidectomy and I^{131} ablation for thyroid carcinoma. After tracer dose of 3 mC I^{131}, uptake measured with wide-angle scintillation counter was less than 1 % and did not significantly differ from body background. Scintillography revealed, however, a definite localized uptake in neck probably due to remnants of normal thyroid tissue.

of radiocolloids (30) and the gallbladder after the administration of I^{131} labelled organic compounds excreted with the bile (31). Brain tumours show, due to the disappearance of the s. c. blood-brain barrier, an increased accumulation of several labelled compounds after intravenous injection (3, 7, 10, 25); this can be used for the detection and localization of the tumour. For this purpose, the most promising technique has been the use of positron-emitters and registration of the annihilation photons by two coincidence-coupled scintillation counters (7).

The localization of radioisotopes *in vivo* is still a relatively young technique, and its medical applications will probably be extended in the future. The apparatus described has been found valuable in clinical routine as well as for research purposes in cases where the localization of gamma-emitting isotopes *in vivo* may yield information of interest.

Acknowledgment

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SUMMARY

A motor-driven directional scintillation counter with automatic recording for the localization of γ-emitting radioisotopes *in vivo* is described.

ZUSAMMENFASSUNG

Ein motorgetriebener Scintillationsdetektor mit automatischer Registrierung für die Lokalisation *in vivo* von γ-emittierenden Radioisotopen wird beschrieben.

RÉSUMÉ

Les auteurs décrivent un compteur à scintillation directionnel à déplacement automatique et enregistrement automatique pour la localisation *in vivo* de radio-isotopes à émission gamma.

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MALIGNANT GRANULOMA

by

S. Hultberg, Hj. Koch, G. Moberger and *G. Mårtensson*

Malignant granuloma, lethal granuloma, granuloma gangraenescens, and osteomyelitis necroticans faciei, are different synonyms for a necrotic process localized to the soft tissues of the face and underlying bone and sinuses, the hard and soft palate, and the pharynx. The disease is well characterized both clinically and patho-anatomically; the course usually prolonged and progressive, terminating in death. Various etiologic considerations were discussed in an earlier paper (BERGQVIST & KOCH 1949). At that time it was chiefly two different etiologic factors that had been suggested in the literature: an inflammatory process of unknown nature, or a malignant neoplasm. The treatment in those days accordingly consisted of anti-infective agents, chiefly antibiotics, or roentgen irradiation in tumor doses. In some cases extensive and disfiguring surgical measures were employed. The results of these therapeutic methods were exceptionally poor; healing was obtained in only a few cases.

In recent years entirely new considerations have emerged, and the possibility has been suggested of an allergic etiology. HOOVER (1941) called attention to the histologic resemblance with the phenomenon of Arthus. WEINBERG (1946) showed that these lesions were not strictly limited to the midline tissues of the face, but that here and there throughout the body a pathologic picture of periarteritis nodosa developed. TEILUM (1946) wrote that "by looking on these lesions from a common pathogenetic point of view, based on morphologic observations and experimental studies on the vascular and tissue changes in allergy, essential support has been obtained for the concept that these lesions are primarily vascular, and are of allergic nature". KLINGE (1933) had earlier stated that periarteritis nodosa was an allergic vascular lesion.

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Since there was justification, it was felt, to assign malignant granuloma to the collagenoses, and accordingly to assume the possibility of an allergic etiology, ACTH and cortisone were tried therapeutically. The literature includes a number of papers reporting this treatment of malignant granuloma, with varying results. WILLIAMS (1949) described a case in which cortisone apparently had a salutary effect. MOORE et coll. (1951) acquired similar experience in a case with lesions involving the nasal cavity, nasopharynx, and larynx, as well as papulopustulous lesions of the elbows, thumbs, fingers, and feet. Biopsies revealed necrosing vasculitis, which was attributed to local hypersensitivity. Cortisone was therefore tried, the patient receiving 200 mg daily for two days, then 100 mg daily for three weeks, and finally 50 mg daily for 14 days. Under this treatment a considerable improvement occurred. On withdrawal of the cortisone therapy the lesions very soon returned, but they regressed again as soon as the treatment was resumed. Several attempts to withdraw cortisone resulted in immediate recurrences. HAGENS et coll. (1953) reported a case with ulcerative lesions at the root of the nose and in the nasal cavity. Biopsy showed "subacute inflammation of fibrous connective tissue" ... "the inflammatory cells consist for the most part of large mononuclear leucocytes, but there are scattered neutrophilic leucocytes and numerous eosinophils as well as a moderate number of plasma cells". Since the condition did not respond to various forms of treatment, including different antibiotics, corticotropin therapy was instituted; 100 mg daily for five days, 75 mg daily for twelve days, and 50 mg daily for eight days, after which the drug was withdrawn. The lesions had healed after 3 to 4 weeks, and there were no signs of recurrence at follow-up seven months later. ALEXANDER (1954) had the opposite experience with ACTH treatment. His patient, who presented extensive lesions in the nasal cavity and had a temperature varying between 37.5 and 40.5° C, was first treated with ACTH 40 I. U. daily for six days. The temperature fell but the lesions continued to spread. ALEXANDER then changed to cortisone, 150 mg daily, whereupon the temperature once more rose to 39 to 40° C and the lesions increased. After seven days he reverted to ACTH and raised the dose to 60 I. U. daily, with the same good effect on the temperature but no effect on the nasal lesions. Despite continued administration of ACTH, the patient died.

BRECKENRIDGE et coll. (1954) reported five cases of malignant granuloma, one of which received ACTH without any effect on the facial lesions. The dosage was not stated. HOWELLS (1955) communicated two cases in which cortisone therapy had good results. These cases were more or less identical: malignant granuloma of the paranasal sinuses, chiefly the ethmoid, with involvement of the orbit resulting in protrusion. Both patients were subjected to evisceration of the orbit, and both received

cortisone. In one of them the dose was 100 mg daily, but unfortunately the duration of treatment was not stated. The other received 100 mg daily for four days, 75 mg daily for three days and 50 mg daily for four days. Radiotherapy was given in each case: 1,500 r over 45 days in one and 1,337 r over 50 days in the other — *i. e.*, small doses. Howells' cases presented histologic lesions suggestive of periarteritis nodosa. "It is possible", he pointed out, "that we are dealing with a general condition in which the local ulceration and granulation is an expression of a hypersensitivity, perhaps precipitated by some factor which may be overlooked in the history. In this condition trauma may possibly be of importance."

GLASS (1955) reported four cases of malignant granuloma. The first patient, who had lesions of the lip, nose and palate, received treatment including ACTH (dose not stated), which arrested the process. However, as soon as ACTH was withdrawn, spread of the lesions continued. Roentgen therapy was then resorted to, instead, in low doses (1,520 r over more than one year), and had a good effect. The second patient was treated initially with roentgen irradiation, which had an excellent effect. Later on, however, there was a severe recurrence, the patient dying after a short time notwithstanding roentgen therapy, aureomycin, and ACTH (dose not stated).

The literature accordingly reports varying results with regard to the treatment of malignant granuloma with ACTH and cortisone, with or without radiotherapy.

In the past seven years ten patients with the diagnosis of malignant granuloma have been admitted to Radiumhemmet and the Ear, Nose and Throat Department of Karolinska Sjukhuset. Eight of them were males and two females, the ages ranging from 27 to 81 years.

Since, as the aforegoing review indicates, new aspects of this disease have emerged, all histopathologic preparations from the above series have been re-examined. The problem has been accentuated in the last year by the admission to Radiumhemmet and the Ear, Nose and Throat Department, of a further four patients with malignant granuloma.

Case 1. K. S. Farmer, aged 27, who two months before admission had increasing pain and swelling in the left nasal ala and upper lip. On admission in October 1948, he had a marked, reddened swelling of the upper lip with a necrosis of the left ala (Fig. 1), necrotic lesions in the left nasal cavity and anteriorly in the hard palate. His general condition was good and blood picture normal. Bacteriologic examination revealed growth of *Staphylococcus aureus*, hemolytic streptococci, and *Escherichia coli*. Serologic findings were negative. Biopsy showed ulcerative inflammatory lesions of malignant granuloma type (Figs. 3 to 7). Patient was treated with a number of antibiotics locally and parenterally (penicillin, streptomycin, tyrothricin). He also received local iodine, gentian violet, oxygen and methylene blue, but without effect. In addition roentgen therapy was given, one month after admission, in a total dose of 500 r in eight treatments over 11 days. This was associated with a conspicuous local improvement: The necroses

Fig. 1. Case 1. The patient on admission.

Fig. 2. Same patient seven weeks later.

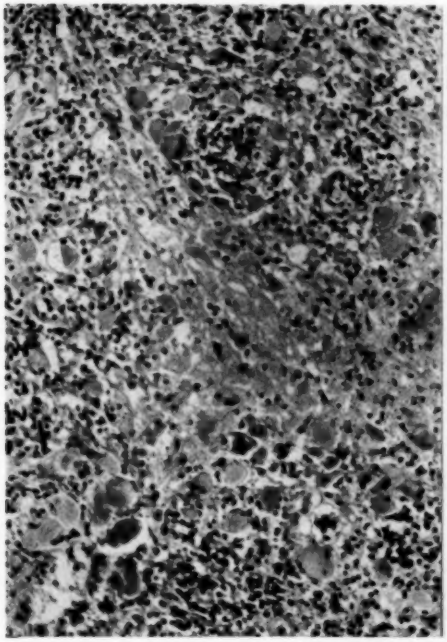

Fig. 3. Case 1. Primary infiltrate with serofibrinous exudate and inflammatory cell infiltration. × 125.

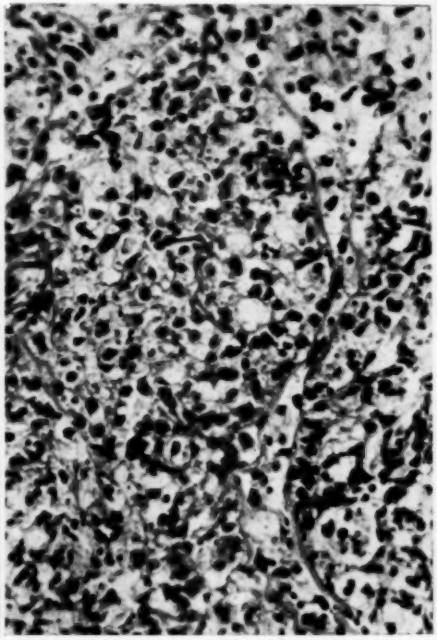

Fig. 4. Same case. Granuloma tissue with a polymorphocellular lymphoreticular structure. × 300.

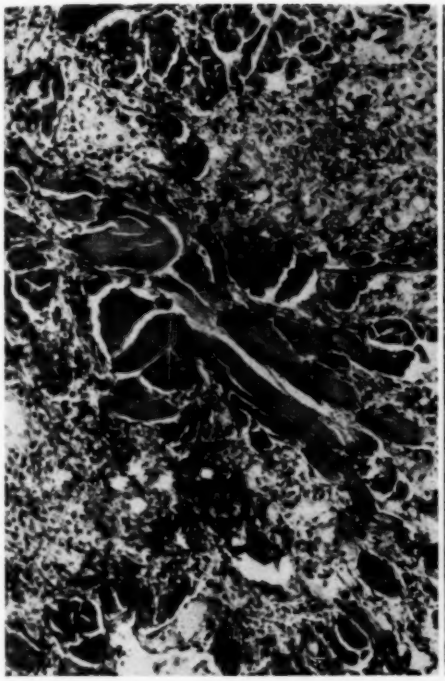

Fig. 5. Case 1. Fibrinoid degeneration of collagenous bundles with hyalinization and disruption (reddish-violet blue in sections stained according to Mallory). × 125.

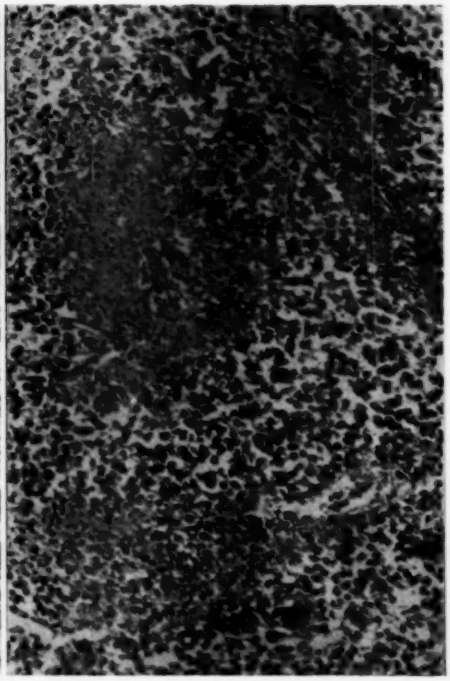

Fig. 6. Same case. Granulomatous cell proliferation in a cervical lymph node with fibrinoid necroses. × 125.

of the face and maxilla, which by then were exceptionally extensive, cleared up and the ulcer margins were clean and healthy. Patient, who had been hyperpyretic, became afebrile. The improvement lasted only one week, after which the high fever returned. Infiltrations the size of the thumb-end developed at several sites in the skin (Fig. 2). Patient's condition rapidly deteriorated and he died, two months after admission, with severe cachexia. (For detailed report, *vide* BERGQUIST and KOCH.)

Case 2. K. S. Female, aged 42, with periodical nasal symptoms of about 10 years standing which had been regarded as allergic. Antiallergic treatment produced no improvement. The nasal distress increased and was very severe in the spring of 1949, when she was admitted to a provincial hospital. Examination disclosed swollen granulating nasal mucosa and a mucopurulent secretion. Hematologic, bacteriologic, and serologic examinations showed nothing noteworthy. At biopsy a peculiar histologic picture was found, mostly resembling granuloma (Fig. 8). There were numerous histiocytes and mononuclear cells together with proliferating fibroblasts and abundant leukocytes, including quite a large number of eosinophils. There was considerable serous exudation and, towards the surface, fibrinoid necrosis as well as widespread ulcerations. In some areas the cell in-

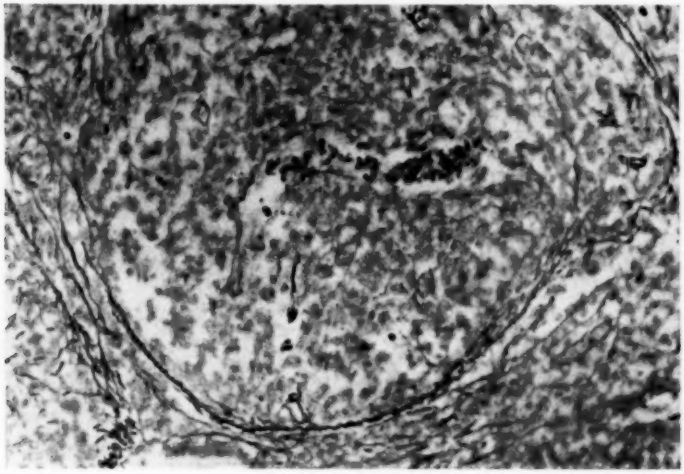

Fig. 7. Case 1. Granuloma tissue in a cervical lymph node. Silver impregnation by Laidlow's method. No demonstrable argentophilic reticulum.

filtrations were of lymphoreticular type, though without demonstrable intracellular argentophilic reticulum (Fig. 9).

Repeated biopsies showed similar lesions. No reliable evaluation of their nature was possible, but malignant granuloma was considered likely. In the differential diagnosis a malignant lymphomatous process was discussed.

Patient showed a temporary improvement after local and parenteral administration of antibiotics (penicillin, streptomycin and sulfa compounds), but soon developed increasingly progressive lesions in the nose, invading the paranasal sinuses, soft tissues of the face, and the soft palate. She was subfebrile. When admitted to the Ear Department of Karolinska Sjukhuset in December 1949 she presented extensive necrotic lesions in the nasal cavity and soft palate. The white blood picture and sternal punctate were normal. Bacteriologic examination revealed growth of *B. proteus*. No destructive lesions were detectable roentgenographically in the facial bones. Antibiotics (penicillin, streptomycin, aureomycin) were administered in large doses without effect. Radiotherapy was tried in small doses (total of 500 to 700 r to each of four fields over two weeks), and produced a transient local improvement. Further administration of antibiotics and roentgen irradiation in small doses had no result. The necroses continued to increase and patient had mounting fatigue and anorexia; then leukopenia. There was remittent pyrexia. Cachexia supervened and patient died in April 1950.

Histologic examination showed, both in the partially thickened mucosa of the nose and paranasal sinuses (specimens from the inferior concha and ethmoid sinus), massive and diffuse cell infiltrations, resembling in principle those observed in previous biopsies. The cytologic picture, however, was somewhat divergent in that the infiltrations consisted of fairly homogeneous, somewhat polymorphic, round or polygonal cells forming a rather fine network. There were fairly numerous mitoses. Groups of mucous glands were enclosed

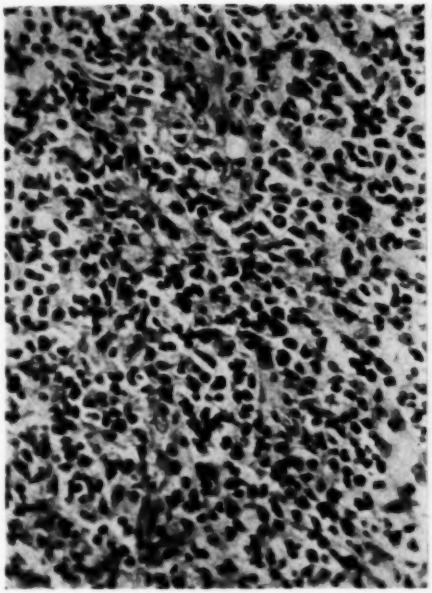

Fig. 8. Case 2. Granuloma tissue from the nasal mucosa, with copious inflammatory cell infiltration. × 300.

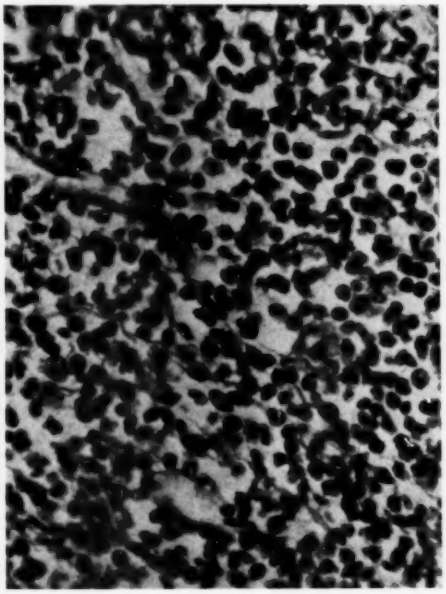

Fig. 9. Same case as in Fig. 8, silver impregnation by Laidlow's method. No demonstrable argentophilic reticulum. × 300.

in the infiltrations, which were also observed invading and destroying the spongy bone in both the inferior concha and the sinus walls (ethmoid). Although the necrosing tendency was but little pronounced, the greater part of the mucosal surface was ulcerated. At the site of a defect in the epiglottic area there was massive cell infiltration of the same type, extending into the mucosa, much of which was ulcerated. In the deeper layers the infiltration was invading and destroying the hyaline cartilage. Throughout the small cervical lymph nodes and superior mediastinal nodes were diffuse areas of tumorous polymorphocellular proliferation, totally obliterating the normal parenchymal configuration (Fig. 10). In contrast to the original infiltrations in the nasal mucosa, a fine, distinct, partially intracellular argentophilic reticulum (Fig. 11) was demonstrable in some of these lymph nodes. In the mesenteric nodes the parenchyma was intact and showed no cell infiltration of similar type. Foci in the left lung corresponded to bronchopneumonic infiltrations with abscess formation. No atypical cell proliferation was detectable therein. The splenic pulp exhibited diffuse areas of tumorous polymorphocellular tissue of similar type to that in the cervical and mediastinal lymph nodes. In the liver, kidneys, adrenals, and hypophysis were scattered infiltrations of corresponding type. A necrotic focus in the uterine wall showed sparse persistent residue of the same process, and polymorphocellular vegetations filled the lumina of several vessels. The findings suggested that the necrotic focus was of a metastatic tumorous character.

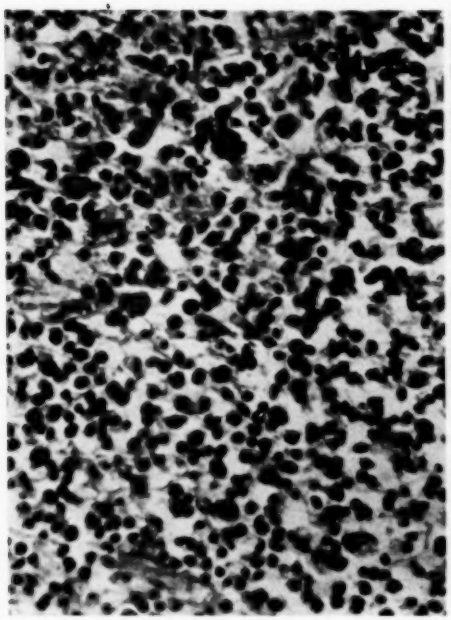

Fig. 10. Case 2. Polymorphocellular infiltration in a mediastinal lymph node. × 300.

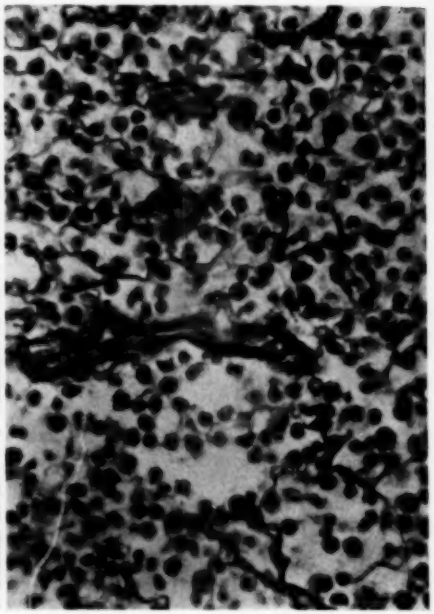

Fig. 11. Same case as in Fig. 10, silver impregnation by Laidlow's method. Abundant reticulum, in part intracellular. × 300.

The cell infiltrations in the nasal mucosa, hypopharynx and epiglottis, cervical and mediastinal lymph nodes, liver, spleen, kidneys, adrenals, and hypophysis were unquestionably of a tumorous character. The cytologic picture was consistent with a malignant lymphomatous process, mostly resembling a poorly differentiated reticulum cell sarcoma. The findings suggested generalization of the malignant process, originating from local infiltrations in the nasal and pharyngeal mucosa, and not a primary systemic disease of leukemic type. The histologic examination yielded no evidence of lymphogranulomatosis or a malignant tumor of other character. Nor did the lesions provide any support for the assumption of a specific inflammatory process.

On comparison of the findings in the autopsy specimens and the biopsies, the cell infiltrations in the former showed a different structure: They presented a more homogeneous and reticular cytologic picture, and in addition argentophilic reticulum was demonstrable. The more granuloma-like tissue from the original infiltrations in the nasal mucosa, together with the absence of argentophilic reticulum, suggested malignant transformation of the process. The histologic findings may thus point to transition of malignant granuloma, not demonstrably of tumorous character, into a malignant lymphomatous process with a cytologic picture and an extension corresponding to poorly differentiated reticulum cell sarcoma.

Case 3. Male nurse, aged 55, who for three years had suffered from increasing nasal obstruction, evil-smelling secretion from the nose, and ulcerations of the hard palate. On admission he presented extensive ulcerations of the nasal cavity with almost total perforation of the septum. In the hard palate was an ulceration about 1.5 cm in diameter, exposing discolored bone and a small perforation to the nasal cavity. The uvula was swollen, and greyish necrosis extended distally across the left tonsil and posterior palatal arch. Biopsy showed a picture typical of malignant granuloma. The W. R. was negative, the red and white blood picture and sternal punctate normal. Bacteriologic examination disclosed profuse growth of *Staph. aureus.* Serologic tests were negative. Treatment consisted of antibiotics (aureomycin and terramycin) and corticotrophin, 40 I. U. for eight days, 20 I. U. for 30 days, and 40 I. U. for 20 days, as well as roentgen therapy with 2,000 r to each of four facial fields in daily doses of 250 to 300 r for five weeks. Irradiation was given (as in all cases reported here) with a 0.5 mm Cu filter; 170 kV and 10 to 15 mA; 50 to 60 cm F. S. D.; H. V. L. 0.7 to 0.9 mm Cu; and with an intensity of 40 to 80 r/min. Under this treatment the ulcerations cleared up, the margins levelled, and the size decreased. Infiltrations then gradually developed at several places in the skin, and enlarged lymph nodes were found here and there. Biopsy showed a picture similar to that in the nose and palate. Patient died six months after admission with general cachexia.

Case 4. K. S. Laborer, aged 45, who had been distressed by nasal blockage and evil-smelling secretion from the left nasal cavity. On admission in April 1952, he presented widespread necroses in that cavity, though the bone was not exposed. Roentgen examination showed no destructive bone lesions. The W. R. was negative; the blood picture normal. Bacteriologic examination revealed growth of *B. proteus.* The biopsy findings indicated malignant granuloma (Fig. 12).

Patient was treated with aureomycin and corticotropin 40 I. U. for 32 days. Radiotherapy was instituted concurrently, with 2,000 r to each of four facial fields, in daily doses of 250 r over six weeks.

Patient's condition greatly improved and he put on weight. The nasal lesions healed nicely. The nasal cavity has since been normal at regular follow-ups.

Case 5. Schoolmistress, aged 52, with marked hereditary allergia. She had been under treatment for asthma and allergic rhinitis since 1940. In 1946 she was treated by resection of the nasal septum, but this resulted in gangrenous dissolution, the root of the nose collapsing. The nose was reconstructed by plastic surgery. Patient had severe bilateral frontal sinusitis and in 1948 underwent an operation on both maxillary sinuses, ethmoidectomy and sphenotomy. However, local disintegration of the tissues in the nasal cavity gradually developed. Biopsy of the nasal mucosa showed a partially necrotizing granuloma-like inflammatory process with heavy serous exudation and numerous eosinophilic leukocytes. Around small focal necroses were observed swollen cells resembling fibroblasts, to some extent at the margins. There was intimal proliferation in small blood vessels, and perivascular inflammatory cell infiltrations, with a preponderance of leukocytes. The pathologic process was not of a tumorous character.

The lesions showed increasing progression and eventually invaded the right orbit. Treatment with ACTH and cortisone at another hospital had arrested the destructive process to some degree, but had not healed it. Patient was therefore referred to Radiumhemmet in February 1952. She received cortisone 25 mg $\times$ 2 and roentgen therapy in a dose of 300 r $\times$ 8 to each of four facial fields between 18 February and 26 March. There was a substantial improvement of the lesions in the right nasal cavity. Since ulcerations were still present in the left ethmoid region, radium was applied locally, the lesions then clearing up and showing incipient healing. In March 1953 there was a severe exacerbation, with pain in the right eye and progressive ulceration of the eyeball. In order to prevent sympathetic ophthalmia, and since patient was in any case blind in the

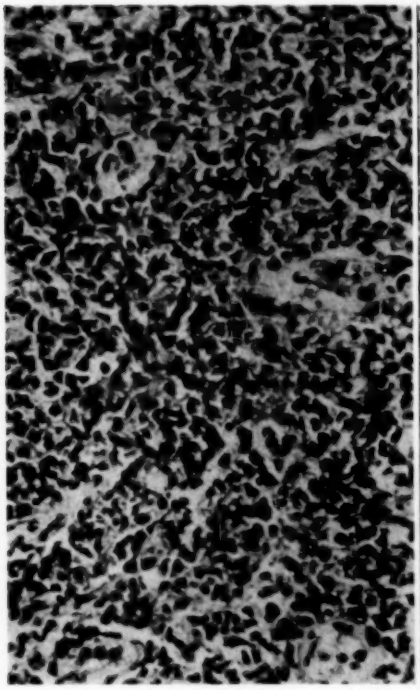

Fig. 12. Case 4. Granuloma tissue with variegated cytologic picture of lymphoreticular type. × 300.

Fig. 13. Case 5. Eyeball infiltrations with copious serofibrinous exudation. × 125.

one eye, evisceration of the right orbit was undertaken in May 1953. The histologic findings were as follows: A greatly contracted and fibrous eyeball, showing a destructive, necrotizing granuloma-like process having a cytologic picture consistent with malignant granuloma. The process was invading the sclera and obliterating the greater part of both the choroid and retina. In it were observed large infiltrations of inflammatory cells, including very profuse eosinophilic leukocytes (Fig. 13). Many of these infiltrations showed perivascular grouping, and in the vessels were intimal proliferation and degenerative changes, imparting some resemblance to periarteritis nodosa (Fig. 14). There was also considerable serofibrinous exudation. The granuloma-like process was not tumorous.

Patient recovered considerably after the operation; her strength increased and she put on weight. The wound cavity cleared up. However, symptoms soon appeared in the left eye; there was redness and, in October 1953, a pea-sized ulceration of the conjunctiva.

She received ten subconjunctival injections of cortisone in the left eye, and beta-irradiation with 500 rep daily to a total dose of 3,000 rep, with the aid of a strontium applicator (Sr^{90}). The ocular lesions subsided within a month or so. One year later there

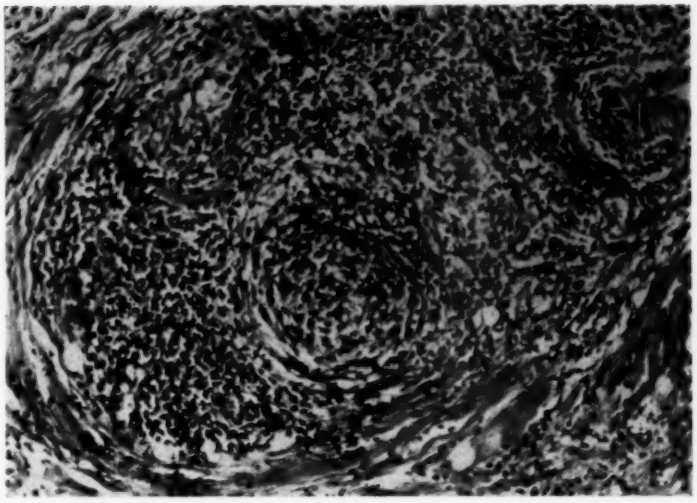

Fig. 14. Case 5. Granuloma tissue from eyeball, showing perivascular inflammatory cell infiltrations and intimal proliferation. × 125.

was a further recurrence, with ulceration of the left eye. She was then given an increased dose of cortisone with 100 mg daily for 2 to 3 weeks, and once more 6 × 500 rep with a strontium applicator, as well as local administration of cortisone ointment. The lesion once more healed about one month after the irradiation.

In March 1955 a small ulceration developed on the conjunctiva, adjacent to the site of the previous ulcerations. The earlier combination of cortisone and beta-radiotherapy was repeated. This time healing took longer and was not complete until the dose of cortisone had been increased to 100 to 150 mg daily for a few weeks.

At a follow-up in August 1955, the right orbit showed complete epithelialization, the left eye had healed, and the nasal cavity was epithelialized. Patient was fitted with a very satisfactory ocular prothesis and was able to resume her work as a teacher.

For the time being she is receiving a dose of 100 to 150 mg hydrocortisone daily, together with 20 I. U. acton prolongatum every 14 days. Notwithstanding cortisone medication for several years, the adrenal function is normal.

Case 6. Male, aged 68, with nasal obstruction of 4 to 5 years' standing, who sought treatment at the Outpatient Ear Department for a swelling in the alveolar process of the anterior part of the maxilla. He also complained of continuous dull pain over that region, as well as severe tenderness on pressure.

Examination disclosed ulcerative necrosis of the gingiva above the incisors and extending across the anterior portion of the hard palate. The ulceration involved the tissues to a depth of about 1 cm, exposing the bone (Fig. 15). The nasal mucosa was so hyperplastic as to cause almost total blockage.

The W. R. was negative and the blood picture normal.

A biopsy from the necrotic area in the alveolar process showed the following findings: An ulcerative and necrotizing granulation-like proliferative process, with severe edema

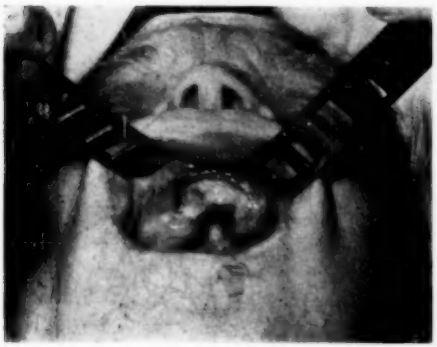

Fig. 15. Case 6. The patient on admission.

Fig. 16. Same case. Granuloma tissue with pronounced inflammatory cell infiltration partly of perivascular type. × 125.

and inflammatory cell infiltration, partly in the form of abscess-like aggregations of leukocytes. The inflammatory infiltration had, in part, the character of perivasculitis (Fig. 16); in addition fibrinoid degeneration both of vessel walls and of collagenous bundles was observed. There were fairly numerous eosinophilic leukocytes. The process did not have a tumorous character, the findings being consistent with granuloma gangraenescens. The histologic and clinical pictures together pointed to malignant granuloma. For this reason combined treatment was instituted, with cortisone in the following dosage: 100 mg cortisone daily for one week; 50 mg daily for 10 days; 40 mg daily for five days; 25 mg for eleven days; and 20 I. U. ACTH in a depot every two days for three weeks. Roentgen therapy was given between 8 April and 8 May 1954, over four facial fields, each with a total dose of 2,500 r.

Patient's condition improved considerably, and after three months the severely necrotic ulcerations had cleared up. At regular follow-ups no local progression was observed. However, three months later a swelling developed round the left eye, with a moist ulceration at the medial angle. Biopsy from the area showed malignant granuloma.

Cortisone therapy was once more instituted, with 100 mg daily for one week. Coincidently, supplementary roentgen therapy was given in a dosage of 200 r × 5 to the ulceration. Following a temporary improvement there was rapid progression, patient dying in April 1955.

Case 7. K. S. Merchant, aged 55. The initial signs appeared in the spring of 1955, consisting of tonsillar necroses that were regarded as Vincent's angina and showed some

response to penicillin and chloromycetin. The process gradually spread to the anterior palatal arches, and in addition fresh necroses developed in the maxillary alveolar process. On admission in July 1955, the alveolar process showed several extensive necrotic areas with exposed bone. The W. R. was negative, the S. R. and blood picture normal. Bacteriologic examinations repeatedly showed growth of *alpha-hemolysing streptococci*, *E. coli* and *Staph. aureus*. Biopsy disclosed proliferating granulation tissue consistent with malignant granuloma. Patient received roentgen therapy, with 2,100 r to each of four fields in daily doses of 300 r over four weeks. In addition, cortisone was administered in an initial dose of 200 mg daily, which was reduced, during the course of one week, to 75 mg daily. Since the lesions continued to spread in the oral cavity and alveolar process, the cortisone dose was raised to 200 mg daily, with a conspicuous improvement in patient's condition. Three months after admission the pharyngeal lesions had regressed and the necrotic ulcerations of the alveolar process had cleared up. Tentative exchange of the cortisone for equivalent doses of deltacortone resulted in a rapid local deterioration; but there was a short improvement after cortisone had been reverted to. Notwithstanding a further increase of the cortisone dose to 250 mg daily and roentgen irradiation with 1,500 r, there were increasing destruction of the entire maxilla, widespread necroses of the mandible, and extensive defects in the soft tissues of the face. Efforts to treat patient with broad spectrum antibiotics and hyposensitization with autovaccine were abortive. Patient died, eight months after admission, from a perforating gastric ulcer.

Case 8. K. S. Farmer, aged 37, with small vesicles and ulcerations on the inside of the lips and the tongue for three years, which had been regarded as aphthous stomatitis. He had been treated with various antibiotics, neoarsphenamine and vaccine, without effect. On admission in July 1955 he presented widespread ulcerations in the oral cavity and pharynx. On the right tonsil and round the uvula was an ulceration extending into the hypopharynx to a point 2 cm above the aryepiglottic region. Similar lesions were observed medially in the soft palate and at the left margin of the tongue. The W. R. was negative, the S. R., blood picture, and temperature normal. Numerous repeated bacteriologic examinations revealed growth of *Staph. aureus*, which after a short time were resistant to a large number of antibiotics. Biopsy showed an ulcerative inflammatory process of malignant granuloma type.

Patient was treated with cortisone in an initial dose of 200 mg daily, which was reduced during the course of one week to 75 mg daily. In addition he received roentgen therapy for three weeks in a total of 2,400 r to each of two fields. The oral and pharyngeal lesions rapidly regressed under this treatment, leaving greyish-white epithelialized scars. A week or so after the dose had been reduced to 75 mg daily, patient had a slight recurrence in the left tonsil, but it speedily subsided when the dosage was raised to 200 mg daily. A further attempt to reduce the dose to 150 mg daily promptly resulted in a fresh ulceration of the posterior pharyngeal wall. When the cortisone dose was once more increased to 200 mg daily, there was first a regression of the pharyngeal ulceration, but then it gradually increased again. Raising of the dose to 250 mg daily produced no further improvement. Supplementary roentgen therapy was given, first with 1,000 r over one week and, one month later, with 2,400 r over two weeks; and in addition local strontium treatment. In spite of these measures the necrosis continued to spread and, five months after admission, involved an area corresponding to the meso- and hypopharynx down to the level of the aryepiglottic region, and extending laterally to the posterior palatal arches. Throughout this time, patient's general condition was exceptionally good. The red blood picture showed slight anemia; white blood picture and S. R. normal. Following very careful removal of loose necrotic tissue, patient became hyperpyretic and had such severe dysphagia that gastrostomy had to be resorted to. Bacteriologic examination showed growth of *Staph. aureus*, resistant to a large number of antibiotics. Tetracyn was then instituted and, concurrently, patient was hyposensitized with autovaccine. During the

next few months a considerable improvement occurred; the large necrotic area in the pharynx cleared up and epithelialized so that only a pea-sized necrosis eventually remained in the hypopharynx. Eight months after admission, however, patient had massive hemorrhage and died.

Autopsy disclosed a necrotic area in the right piriform sinus and rupture of the superior thyroid artery.

Case 9. RH. Compositor, aged 61, who, about one week before admission to Radiumhemmet, had had pain round the left lower third molar and the right upper second premolar and first molar. On admission he presented a gangrenous process involving a centimeter-wide zone in the gingiva round the loose left lower third molar. In the buccal mucosa at that site was a superficial ulceration with a necrotic surface. Similar lesions were observed around the two above-mentioned maxillary teeth. Biopsy disclosed necrotizing inflammatory process having a histologic picture consistent with malignant granuloma.

Cortisone therapy was instituted in a dose of 80 mg daily, and in addition roentgen therapy with 2,000 r to each of two fields over three weeks. This was followed by a distinct improvement of the lesions on the left side but, if anything, slight worsening of those on the right. For this reason, a further 500 r was delivered to the right side. One month later the lesions had completely subsided. Patient continued to receive cortisone therapy with 20 mg deltacortone daily. At follow-up six months later, he was asymptomatic.

Case 10. RH. Retired mill hand, aged 81, with an ulceration of four months' standing in the anterior maxillary gingiva, which had gradually increased in size. On admission to Radiumhemmet a disintegrating growth, about the size of the thumb-end, was observed buccally in the left maxillary alveolar process, invading the upper lip and floor of the nose on the left side. Bacteriologic examination showed growth of *Staph. aureus*. The blood picture and temperature were normal. Biopsy revealed a non-specific ulcerative inflammatory process histologically resembling malignant granuloma. Patient received local treatment with varidase and chloromycetin topical, and parenteral administration of penicillin (600,000 I. U.) and streptomycin (1 g). Cortisone therapy was initiated with 300 mg daily for three days; then 200 mg daily for one month. Acton prolongatum was interposed once weekly in a dose of 100 I. U. Subsequently the medication was changed to deltacortone 50 mg daily, which was reduced after 14 days to 30 mg daily.

Concurrently, roentgen therapy was given in small doses (2,500 r over three weeks).

Under treatment the maxillary necrosis cleared up considerably, and satisfactory demarcation with a healthy margin was obtained. A defect the size of the thumb-end gradually developed in the upper lip, but here too the lesions cleared up and the marginal zone appeared healthy.

Patient, on admission, had been in poor condition due to pronounced cardiac insufficiency; after showing an initial improvement he died four months later. By that time the lesions in the maxilla and upper lip had increased further in extent.

Discussion

The initial process in the malignant granuloma cases presented showed the histopathologic picture of an inflammatory tissue reaction, with the proliferative changes predominating. In each case considerable serous exudation was observed (Figs. 3 and 13), with profuse infiltration of inflammatory cells of both mononuclear (lymphocytes and plasma cells) and polynuclear (leukocytes) type (Figs. 3, 8, and 12). The eosinophilic

leukocyte count varied — in some cases substantially (Cases 4, 5, and 6). The process was characterized by a marked proliferative tendency, chiefly of histiocytes and reticular cell elements, though also of fibroblasts and capillary endothelial cells. This cell proliferation largely had the appearance of a granulation tissue, to which the admixture of inflammatory cells had imparted a variegated cytologic picture (Figs. 4, 8, and 12). In several cases the highly cellular process showed, rather, the picture of a lymphoreticular network (Figs. 4 and 12). However, argentophilic fibrils were not demonstrable (Figs. 7 and 9). The process was further characterized by the major, and frequently extensive, tendency to necrotization of fibrinoid type (Fig. 14), often leading to progressive and deep ulcerations.

In none of the cases reported here was the cell proliferation in the initial lesions demonstrably tumorous. Nor could the primary infiltrations (Figs. 8 and 9) in Case 2 — where the autopsy specimens showed the picture of a malignant tumor, chiefly suggesting reticulum cell sarcoma (Figs. 10 and 11) — be regarded as tumorous in character. In this case the histopathologic findings lent weight to the assumption of malignant transformation.

The frequently reticular character of the initial granuloma-like process may suggest some form of malignant progressive reticulosis. This seems to be disproved by the absence of argentophilic reticula in the cell infiltrations (Figs. 7 and 9), and by the fact that all cases presented local progressive lesions without a systemic character. Nor did the histopathologic findings afford any evidence of a process of leukemic nature. In those cases where bone marrow was examined, the cytologic findings were largely normal — if anything showing slight signs of hypoplasia. The major necrotizing tendency together with a disposition to sclerosis in the late stages (*e. g.*, Cases 1 and 5) might be suggestive of lymphogranulomatosis; but the absence of giant cells and the cytologic picture in other respects provided no support for such an assumption. No lesions of specific inflammatory nature were observed.

Several authors have called attention, on clinical grounds, to the possibility of the granulomatous process being an allergic manifestation. Allergic tissue reactions present the histopathologic picture of a nonspecific inflammatory process. But those morphologic characteristics that are usually detectable in lesions of allergic etiology, such as eosinophilia, perivasculitis, fibrinoid degeneration of vessel walls and collagenous bundles, and fibrinoid necroses, cannot be considered as pathognomonic. Hence it is difficult solely on the basis of the histopathologic findings to deduce with assurance whether an inflammatory process is of allergic origin. However, the presence of such specific lesions as commonly attend allergic conditions, when correlated to the clinical signs and the response to therapy, may point to an allergic etiology.

Such lesions were observed in the majority of the malignant granuloma cases presented. In the primary infiltrations the serous exudation in the tissue was conspicuous. In several cases eosinophilic leukocytes were fairly profuse. The inflammatory cell infiltration in Cases 5 and 6 appreciably resembled perivasculitis, and the histopathologic picture showed major similarities with periarteritis nodosa (Fig. 14). The major tendency to fibrinoid necrosis also was noteworthy in all cases; moreover, it is common in allergic processes. Several of the cases even showed distinct fibrinoid degeneration of collagenous bundles (Fig. 5), often with disruption or fragmentation. The vascular lesions played a dominant role in the histopathologic picture. The tendency to perivascular grouping of the inflammatory cell infiltrations has already been mentioned. Edematous swelling was often observed in the vessel walls themselves, together with more or less pronounced intimal proliferation, frequently leading to obliteration of the lumina. Medial thickening associated with fibrinoid degeneration was observed in numerous vessels within the granulomatous cell proliferations in several cases.

The regularly occurring histopathologic lesions demonstrated here suggest that allergic factors may well be instrumental in the genesis of malignant granuloma.

The most striking feature of the symptomatology is the inconsistency between the grave lesions and the absence of a causative agent. This was first pointed out by GRUBER in the case of periarteritis nodosa, and, together with the tissue eosinophilia, it prompted him to suggest the possibility of an allergic etiology in that disease. KLEMPERER et coll., too, fully subscribes to the view that periarteritis nodosa is an allergic disease.

As regards the allergen, it has been pointed out (COOKE) that bacterial allergy is a common cause of several different allergic diseases, including periarteritis nodosa. The bacteria most commonly encountered are streptococci, pneumococci and staphylococci. WILLIAMS called attention to bacterial allergy as an etiologic factor in malignant granuloma. RICH, in experimental investigations with slightly virulent bacteria in previously immunized animals, produced lesions resembling periarteritis nodosa. In the field of bacterial allergy, special importance has been attached to the staphylococci.

An allergic heredity and allergic manifestations were observed in two of our patients, but there was no history of such in the others.

In one half of our cases the bacteriologic examination showed abundant *Staph. aureus* in a very large number of samples. In three cases it revealed only the usual pharyngeal flora, but in two patients a profusion of *B. proteus*.

Whether *Staph. aureus* played a fundamental role as an allergen cannot be decided with current methods of examination, though it seemed to have done so in one patient (Case 8) in whom it was found in large numbers at each examination. When loose pieces of necrotic tissue were carefully excised, the patient responded with sepsis-like signs associated with hyperpyrexia and a poor general condition, which were combatted with tetracycline. Cultivation of bacteria, disruption of them without destroying or appreciably altering the bacterial protein, and extraction, yielded a vaccine which, when tested on the patient, elicited an immediate wheal. Concurrent administration of tetracycline and vaccine produced exceptionally good local improvement, which in this case had not previously been observed with antibiotics to which his staphylococci were susceptible at the time. Similar attempts to disrupt bacteria (*Staph. aureus*) failed insofar as it was not possible to obtain extracts eliciting an immediate wheal. Trials with ordinary autovaccine in conjunction with antibiotics produced no definite effect.

The observations are thus inconclusive. It would nevertheless seem that the approach outlined above might help to resolve the problem of the pathogenesis of malignant granuloma.

Both the literature and our own experience show that only in exceptional cases has malignant granuloma healed solely under radiotherapy or operative treatment. Roentgen irradiation has very occasionally brought about healing, but here large doses have been delivered as in the treatment of carcinoma. Transient improvements have been effected, but as a rule the gangrenous process has inexorably progressed and caused death.

With ACTH and cortisone it has been possible to arrest the necrotizing lesions for a time but not to heal them. Only a combination of irradiation and adequate cortisone therapy may, it would seem, yield better results. This is nicely demonstrated in our Case 5.

That cortisone does produce an effect in malignant granuloma seems certain. In all of our patients we observed some effect on the lesions, albeit temporary in some cases. On several occasions the lesions were seen to increase on reduction of the dose, but raising it produced an improvement. Under continuous cortisone medication one patient was kept asymptomatic, and in another the cortisone was withdrawn, without a recurrence, after the lesions had healed, the observation period being five years.

In other cases, again, cortisone was of very limited value. It was possible to arrest the lesions or greatly to reduce them temporarily. In due course the process became refractory to cortisone and not even very large doses produced any effect.

As regards radiotherapy, it is probably necessary to deliver fairly

large doses. In localization to the nasal cavity and maxilla, a tumor dose of 4,000 to 5,000 r over about four weeks is to be recommended. Here we used 170 kV, 15 mA; 50 to 60 cm F. S. D.; filter 0.5 mm Cu, and an intensity of 40 to 80 r/min; H. V. L. 0.7 to 0.9 mm Cu; daily dose 250 to 350 r, measured on the skin, secondary radiation included. In the earlier cases where we had only given absorption irradiation in small doses, the effect was unsatisfactory.

In several cases the radiotherapy helped to produce at least a temporary improvement. There are various conceivable explanations. In radiotherapy of local allergic conditions as, for instance, allergic rhinitis and bronchial asthma, the frequently exceptional, though temporary improvement has been attributed to local tissue dissolution resulting from the irradiation and in turn giving rise to local hyposensitization.

The roentgen therapy produces general dilatation of the capillaries in the irradiated area. In malignant granuloma, where there is a vascular lesion resembling periarteritis and associated with more or less pronounced constriction of small vessels, roentgen irradiation causes dilatation and an increased blood flow, which in turn should produce the local improvement.

Yet it is also important that cortisone be administered in adequate doses and for a sufficient length of time. Two of our patients are instructive examples of this. Attempts to obtain results with low doses of cortisone repeatedly led to recurrences or exacerbation of the morbid process, which regressed again when the dose was increased.

SUMMARY

Ten cases of malignant granuloma are reported. The careful histopathologic examinations performed in all the cases give support to the assumption that the condition may be an allergic manifestation, and *Staphylococcus aureus* is discussed as an allergen. The value of cortisone therapy is described and emphasized and the effect of roentgen treatment discussed.

ZUSAMMENFASSUNG

Zehn Fälle von malignem Granulom werden berichtet. Die sorgfältigen patologisch-anatomischen Untersuchungen, die in allen diesen Fällen durchgeführt wurden, unterstützen die Annahme, dass die Erkrankung eine allergische Manifestation sein könne;

der *Staphylococcus aureus* wird als ein Allergen diskutiert. Der Wert der Cortisonbehandlung wird beschrieben und besonders betont. Der Effekt der Röntgenbehandlung wird besprochen.

RÉSUMÉ

Les auteurs présentent dix cas de granulome malin. Les examens histopathologiques soigneux faits dans tous les cas viennent à l'appui de l'hypothèse de la nature allergique de cette affection; les auteurs étudient le rôle du staphylocoque doré comme allergène. Ils décrivent et soulignent l'intérêt du traitement par la cortisone et examinent l'effet de la roentgenthérapie.

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A METHOD FOR THE MEASUREMENT OF THE THICKNESS OF HISTOLOGIC PARAFFIN SECTIONS USING THE BACKSCATTERING OF BETA PARTICLES

by

Erik Odeblad

Several methods have been proposed for the measurement of the thickness of histologic sections. A short review, and his own method, have recently been published by HALLÉN (1956). A simple optical method was described by the present author in 1952; in the present method, the backscattering of low-energy beta particles is utilized.

From defined geometry the backscattering of beta particles from pure elements is known to depend upon the atomic number, Z, of the backscattering specimen. This was established as early as 1907 by SCHMIDT, and since that time the same statement has appeared in several publications. If a composite scatterer (for example a chemical compound) is used, the fraction of scattered beta particles is closely related to the average atomic number Z_{av} of the specimen. The relation between Z and the backscattering fraction is not linear (see *e. g.* GLENDELIN and SOLOMON (1950)).

Since Z_{av} for paraffin (2.7) differs considerably from that for glass (~ 9) we have carried out an investigation to determine whether there is a relation between the thickness of paraffin sections mounted on glass and the backscattering of soft beta rays. The existence of a relation between the backscattering fraction and thickness of target was also described by SCHMIDT (1907). The present investigation shows the existence of such a relation for the glass—paraffin system. The relation is of such a magnitude that it may form the basis for the measurement of the thickness of paraffin sections.

The experimental arrangement used is shown in principle in Fig. 1. Deposits of C^{14} or S^{35} were usually used as sources of beta rays, the former

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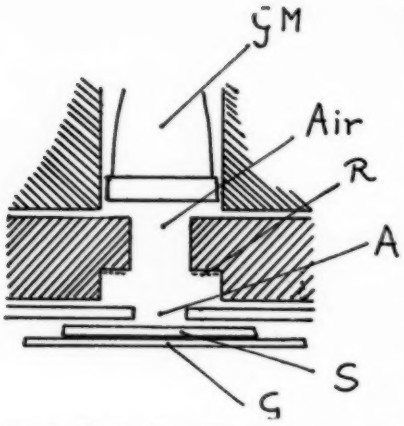

Fig. 1. Schematic outline of arrangement for measurement. R = radioactive source of beta rays. A = aperture in methylmetacrylate plate. S = paraffin section. G = glass slide. GM = Geiger-Müller tube with window, 1.6 mg/cm². Air = air in the chamber; may be replaced with helium gas, if desired. Shadowed areas denote lead shielding.

being found better because of its long life-time. The aperture over the specimen could be changed stepwise between about 0.2 and 2 cm², and the aperture was made of methylmetachrylate in order to obtain as low a backscattering background as possible.

Total backscattering counting rates (including background) were obtained for pure paraffin of 'infinite' thickness (c_p), glass of 'infinite' thickness (c_g) and the unknown or standard specimen (c_x) — a paraffin section on a glass slide. The slight variation in glass geometry which results from the varying thickness of the paraffin layers was considered to be of no importance.

Some results are collected in Diagram 1. It is evident that there is a definite relation between the section thickness and backscattering counting rate and that it satisfies the binominal function

$$B(w) = (1 - w/W)^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

with sufficient accuracy. The binominal function is of the same type as that proposed by ODEBLAD (1955) in describing beta ray absorption or transmission. In eq. (1), w is the thickness of the unknown or standard section and W the limit for 'infinite' thickness. B in eq. (1) is defined as

$$B = (c_x = c_p)/(c_g - c_p) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

From eq. (1) we may arrive at the following expression for the error $\varepsilon(w)$ in w by differentiation:

$$\varepsilon(w) - \varepsilon(B)\ W/n(1 - w/W)^{n-1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Here $\varepsilon(B)$ is the error in B, obtained by applying the common rules of differentiation to eq. (2). The error in any counting rate, c, is governed by the common formula $\sqrt{c/t}$, where t is the counting time. Other errors in addition occur.

For C^{14} beta radiation, the exponent n has the value of 5. In the thickness region 1—4 μ, the error is expected to be < about 10 per cent, provided over 50,000 counts are recorded at each reading of c. With an

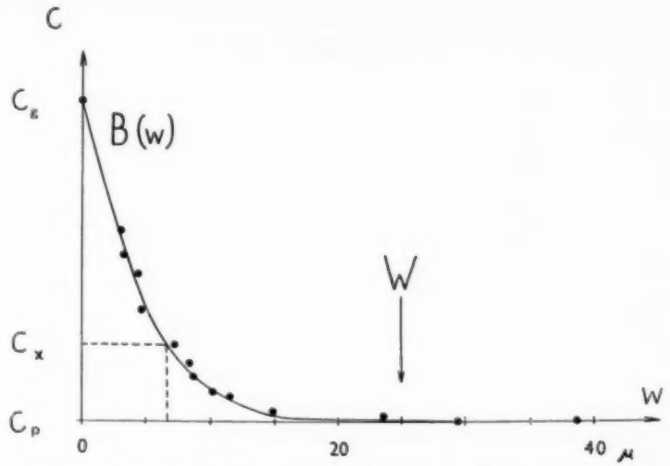

Diagram 1. Typical example of calibration points for a C^{14} source. c = counting rate. c_g = counting rate for glass, c_p = counting rate for paraffin. w = thickness in μ of paraffin section. W = limit of 'infinite' thickness. c_x = counting rate of unknown section, the thickness of which is evaluated as 6.3 μ. The full curve represents function $B(w) = (1 - w/W)^5$. Explanation of $B(w)$ in eq. (2).

increasing thickness of the section the error becomes larger. Sections thicker than 10 μ cannot be measured with a C^{14} beta source; in such cases a Ca^{45} source capable of recording sections up to 25 μ with sufficient accuracy ($<$ 10 per cent error), must be employed (see Table 1).

The errors may in principle be reduced by taking a large number of counts. In the results underlying Diagram 1, and other measurements not reported here, 400,000 counts have been recorded. A drawback then arises in the long counting times necessary. In such cases we have allowed counting rates up to 25,000 per minute with careful corrections for coincidence losses in the Geiger-Müller tube and scaling circuits. Systematic errors may also occur, for example in the preparation and measurement of standard sections; the thickness of these has been controlled by cross-sectioning and weighing.

The measurements described refer to pure paraffin. Histologic sections usually differ in average atomic number, in that Z_{av} is estimated to be about 3.7 for fixed dehydrated tissue of average composition. Since, in the tissue area of a ribbon, about 3 parts out of 4 may be paraffin, Z_{av} in the tissue area of the section may be expected to be about 3.0, which is in agreement with measurements based on 'infinite' specimens of that kind. This implies a systematic error, shifting the values of section thickness some five per cent to a lower section thickness, if reference

Table 1

Information about some pure beta emitters suitable for application in thickness measurements.

Source	Half-life	Max. energy, MeV	Max. range, mg/cm²	Practical range of measurement, μ, paraffin	n
Ni^{63}	85 y	0.063	4	0.3—1 (estimated)	?5
C^{14}	5,600 y	0.15	25	1— 10	5
S^{35}	87 d	0.17	30	1— 10	5
Ca^{45}	152 d	0.25	65	2— 25	4
Cl^{36}	4×10^4 y	0.71	270	5—100	3
P^{32}	14 d	1.70	750	10—250	2

is made to pure paraffin when measuring over the tissue area of ribbon (see Table 2).

One advantage of the method proposed is that it may much more easily be applied to sections mounted on heavier supports than glass, for example on aluminium ($Z = 13$). Aluminium foils are used in activation analysis of histologic sections with charged particles (ODEBLAD 1956). In quantitative autoradiography, especially when applying the refined matrix theory to the calculations, the numerical values of section thickness, obtained with this simple method have also proved to be of value (ODEBLAD 1956). In autoradiography, the amounts of radioactive isotope in the sections are usually negligible compared to the high back-scattering counting rates, but may easily be accounted for in separate countings.

Table 2

Typical counting rates in units of 10^4 counts per minute of some standard specimens.

Specimen	Counting rate
Pure paraffin	1.09
Tissue area of paraffin	1.11
Methylmetacrylate	1.17
Glass	1.52
Aluminium	1.71

Comments: The paraffin is 'histological' paraffin containing 95 parts *w/w* of refined, water-free paraffin of melting point 56° C and 5 parts *w/w* of beeswax. All counting rates were made within one hour so that variations in transmission of beta particles in chamber gas (air), at atmospheric pressure and humidity, were negligible. This factor otherwise has to be considered. The statistical error in each of the tabulated figures is less than 0.01.

Acknowledgment

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SUMMARY

The different degrees of backscattering of beta rays from paraffin and glass are utilized for the measurement of the thickness of histologic sections. The method is simple and gives sufficiently accurate results. Some sources of error are discussed.

ZUSAMMENFASSUNG

Die verschiedenen Graden von Betastrahlen-Rückstrahlung an Paraffin und Glas werden zur Messung der Dicke histologischer Schnitte verwendet. Die Methode ist einfach und gibt zureichend genaue Resultate. Einige Fehlerquellen werden besprochen.

RÉSUMÉ

Pour mesurer l'épaisseur des coupes histologiques l'auteur utilise les différences d'intensité de diffusion rétrograde des rayons bêta par la paraffine et par le verre. Cette méthode est simple et donne des résultats suffisamment exacts. Certaines sources d'erreur sont étudiées.

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BOOK REVIEWS

BIOPHYSICAL INVESTIGATIONS OF URINARY CALCULI. An X-ray crystallographic and microradiographic study. By Curt Lagergren. 71 pp., 33 figs. Acta radiol. Suppl. 133 (1956). Sw. Kr. 25: —.

The mechanism of formation of urinary calculi has long been the subject of intensive investigation, both from theoretical and clinical points of view. One of the many possible routes by which this problem may be approached experimentally is to attain a deeper understanding of the chemical composition, structural topography, and physical orientation of the constituents of urinary calculi. In the present investigation modern refined biophysical techniques have been utilized to define more closely the finer structural organization of these concretions.

The composition of 600 calculi has been determined with aid of a variety of roentgen powder diffraction procedures. The material has been analyzed by the Debye-Scherrer diffraction technique and a short description is given of the preparation of the specimens, the different Debye-Scherrer cameras, and the evaluation of the diffraction patterns. Among the other powder diffraction methods, the Geiger-Müller counter roentgen diffractometer technique has been found very useful, especially for the determination of crystallite size and the quantitative analysis of mixtures. The architecture of concretions was studied by microroentgenography, a procedure which gives both a topographic roentgenogram of the calculus, as well as information about the distribution of its components, depending on their varying absorbing power for roentgen rays. The composition of the different structures seen in the microradiograms was further analysed by using a precise roentgen microdiffraction method which permits investigation of areas of microscopic dimensions.

Eleven crystalline compounds were identified in the 600 calculi examined. The percentage occurrence of these components has been tabulated, and details regarding the mutual relationships of these constituents have been described. The texture of different types of calculi as determined both by microroentgenography and by microdiffraction has been described in detail and is reproduced in pictures. Special attention has been drawn to the fact that in finely-lamellar calculi made up of several crystalline components, individual lamellae consist only of a single component.

Comparison of biophysical and chemical methods has shown that the common methods of chemical analysis are not sufficiently accurate to distinguish between similar substances such as the three calcium phosphates known to occur in calculi. The chemistry, structure, and occurrence of these phosphates in calculi have been discussed in more detail. The interrelationship between basic calcium phosphate (apatite) and calcium carbonate has been re-examined in the light of recently published investigations of surface phenomena of the extremely small apatite crystallites. The size of apatite crystallites in calculi has been found to be about the same as that of other calcified structures of the body.

Consideration has also been given to the significance of the topographic structure of calculi with regard to the known conditions for precipitation of the different crystalline constituents. In some instances it appears that very small alternations in the ionic milieu of the urine may cause the precipitation of two different crystalline components.

Autoreview.

RENAL ANGIOGRAPHY IN EXPERIMENTAL HYDRONEPHROSIS. By Hans Idbohrn. 85 pp., 45 ill. Acta radiol. Suppl. 136 (1956). Price Sw. Kr. 20: —.

In prolonged complete obstruction of the ureter intravenous urography will show no excretion of contrast medium on the side involved. Once the kidney has ceased to function, clinical and urographic examinations are usually of little value in assessing the chances of functional restoration on removal of the obstruction. In such cases renal angiography is the only method available for estimating the extent of parenchymal destruction. Discrepancies are, however, sometimes found between the angiogram, the operative findings or the roentgenograms taken during follow-up. The purpose of the present investigation was to estimate the range of these discrepancies.

A survey of the literature is followed by a description of the material, methods and technique used. Parenchymal destruction was produced by ligation of the ureter. Renal angiograms were taken before ligation of the ureter, during stasis, and after stasis had been relieved by ureterostomy. Changes in the width of the renal artery, in the appearance of the intrarenal arteries, and in the nephrographic effect were studied and are recorded graphically. Renal function was assessed shortly after ureterostomy. The kidneys were afterwards examined macroscopically and microscopically.

An analysis is presented of the angiographic findings of the obstructed side and the thickness of the cortex and the histologic findings are studied for any variation with the duration of stasis.

A similar analysis covers the angiographic findings and renal function after ureterostomy.

The anatomic, functional, and angiographic findings made before and after ureterostomy are likewise compared. These factors are afterwards analysed for any variation with direct measurements of the renal blood flow.

The individual correlations found between various pathologic, functional, and angiographic findings are given graphically and, finally, the angiographic and pathologic changes on the control side are described and discussed.

Autoreview.

ÜBER DIE TECHNIK DER SIMULTANEN TELEFILMPLANIGRAPHIE. Von Vidar Backlund. 125 Seiten, 39 Abb. und 12 Tabellen. Acta radiol. Suppl. 137 (1956). Schwed. Kr. 25: —.

In the introduction a short review of the terminology and literature of the technic of planigraphy are given and in the first chapter the author presents the problems which are to be solved. The method of planigraphy (stratigraphy, laminagraphy, tomography) is of such importance in radio-diagnostics that the existing drawbacks of the technic must be eliminated; most important among these are the high roentgen ray dosage applied to the skin and the time-consuming work involved in serial planigraphy. Another problem is the difficulty of exactly correlating the layers.

The second chapter consists of an analysis of the effect of scattered radiation. The influence of different factors upon the quality of roentgenograms are investigated with a view to enabling exposure rates to be made as small as possible. It was found that a correct relationship between focal distance, focal dimensions, size of the primary diaphragm, and the distance between the object and the film (Groedel—Laurell technic) was most suitable for films up to 35 sq. inches. For larger fields a combination of this technic (telefilm technic) and a high ratio secondary diaphragm of 16 : 1 was best.

In the third chapter the telefilm is adapted to the planigraphic method and compared with the ordinary technic. An analysis of the influence of different arrangements

made with intensifying screens and film emulsions is also included, and from which it is concluded that screens arranged on both sides of the film, which should be double-coated, are to be preferred when high contrast is required. The roentgen ray dosage applied to the skin and the exposure rates are considerably lower in telefilm planigraphy than in the ordinary technic.

The fourth chapter describes the author's method of making planigrams simultaneously, based upon the experiments in the earlier chapters. The layer obtained depends on the level at which the film is situated. A series of films placed behind each other and exposed during a planigraphic movement produce different planigrams simultaneously. Special intensifying screens for the simultaneous technique were constructed in cooperation with a Swedish screen factory. These screens give the same degree of blackening to all planigrams, as well as the same grain size from the first to the eleventh film. The K-radiation from the tungsten screens was diminished with the aid of tin foils.

The cassettes are constructed so that different types of examinations can be made. The leading principle is that all the layers required for a complete examination shall be obtained by one exposure. Thus the cassettes for the examination of the lungs contain 11 layers with a distance of 1 cm between each, the distances being correlated exactly to each other. The different cassette types have layer distances varying from 2.5 to 20 mm, and are made in sizes of 5 $^1/_2$ by 7 $^1/_2$, 7 $^1/_2$ by 10, and 10 by 12 inches. *The roentgen ray dosage measured in simultaneous telefilm planigraphy examinations is only 1 to 10 % of that applied in the ordinary technic.* The work is reduced to a minimum, and even patients in poor fettle may be examined completely when the method is used. Because of the high tube tension, and because single screens must mostly be used, the planigrams obtained are not quite of the same quality as those obtained one by one, but this last drawback is of minor importance.

In the same chapter the use of different kinds of films and developers is discussed as well as a modification of the Siemens Universal planigraph made by the author for greater convenience of the examination. The cassettes and the cassette carrier are adaptable to most of the available planigraphs.

In the fifth and last chapter the author gives some clinical applications and includes exposure data.

Autoreview.

CELLULAR ASPECTS OF BASIC MECHANISMS IN RADIOBIOLOGY. Ed. by H. M. Patt and E. L. Powers. Nuclear Science Series Report Number 18. 190 p. National Academy of Science. Washington 25, D. C., 1956.

The Nuclear Science Series on physical, chemical, and biologic aspects of radiation have the character of transcripts of informal conference discussions on certain basic problems. The number of participants is mostly restricted to 30 or less, representing experts of various disciplines and interests. Five experienced workers are the principal essayists and discussion leaders of this symposium which covers radiation effects on the cellular level. The presentation is vivid, sometimes fascinating, and the discussion can be followed with a fairly ordinary knowledge of cell cytology and genetics.

Arne Forssberg.